PATENT

ATTORNEY DOCKET NUMBER 19425

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent No. 5,820,870	)
Granted: October 13, 1998	)
	)
Patentees: Joseph G. Joyce *et al.*	)
	)
Assignee: Merck & Co., Inc.	)
	)
FOR: RECOMBINANT HUMAN PAPILLOMAVIRUS TYPE 18 VACCINE	)

Commissioner for Patents
U.S. Patent and Trademark Office
Mail Stop Patent Ext.
Randolph Bldg.
401 Dulany Street
Alexandria, VA 22314

APPLICATION FOR EXTENSION OF PATENT TERM PURSUANT TO 35 U.S.C. § 156

Sir:

Pursuant to Section 201(a) of the Drug Price Competition and Patent Term Restoration Act of 1984, 35 U.S.C. § 156(a), Merck & Co., Inc. (hereinafter referred to as "Applicant") hereby requests an extension of the patent term of United States Patent No. 5,820,870 (hereinafter referred to as "United States Patent 5,820,870" or "the '870 Patent").

Applicant represents that they are the record owners of the entire interest in United States Patent No. 5,820,870, by virtue of assignments from the inventors thereof recorded in the United States Patent and Trademark Office (Reel/Frame: 8908/0930) with respect to the patent application leading thereto as documented in **Exhibit 1** hereto.

The undersigned registered practitioner, Joanne Giesser (Reg. no. 32,838) has been authorized to act on behalf of Applicant with respect to this application, and inquiries and correspondence relating to this application are to be directed as set forth in section (15) below.

The following information is submitted in accordance with 35 U.S.C. § 156(d) and 37 C.F.R. § 1.710 et seq., and follows the numerical sequence and format as set forth in 37 C.F.R. § 1.740(a):

(1) A complete identification of the approved product as by appropriate chemical and generic name, physical structure or characteristics;

The approved product is Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine, which is referred to herein by its proprietary name Gardasil®. Gardasil® is a non-infectious recombinant, quadrivalent vaccine prepared from highly purified virus-like particles (VLPs) of the major capsid (L1) protein of HPV Types 6, 11, 16, and 18. The L1 proteins are produced by separate fermentations in recombinant *Saccharomyces cerevisiae* and self-assembled into VLPs. In addition to VLPs, each vaccine dose contains aluminum (as amorphous aluminum hydroxyphosphate sulfate adjuvant), sodium chloride, L-histidine, polysorbate 80, sodium borate, and water.

Gardasil® has been approved by the Food and Drug Administration for vaccination in females from 9 to 26 years of age for prevention of the following diseases caused by Human Papillomavirus (hereinafter HPV) Types 6, 11, 16, and 18:

- Cervical cancer
- Genital warts (condyloma acuminate)
- Cervical adenocarcinoma *in situ* (AIS)
- Cervical intraepithelial neoplasia (CIN) grade 2 and grade 3
- Vulvar intraepithelial neoplasia (VIN) grade 2 and grade 3
- Vaginal intraepithelial neoplasia (VaIN) grade 2 and grade 3
- Cervical intraepithelial neoplasia (CIN) grade 1

See Approved Label attached as **Exhibit 2** with regard to the statements in this Section (1).

(2) A complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred;

Gardasil® (Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine) was subject to regulatory review under Section 351 of the Public Health Service Act (42 U.S.C. §262).

(3) **An identification of the date on which the product received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period occurred;**

Gardasil® (Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine) received permission for commercial marketing or use under Section 351 of the Public Health Service Act (42 U.S.C. §262) upon approval of BLA, STN BL 125126/0 on June 8, 2006. A copy of the FDA approval letter is attached as **Exhibit 3**.

(4) **In the case of a drug product, an identification of each active ingredient in the product and as to each active ingredient, a statement that it has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, or a statement of when the active ingredient was approved for commercial marketing or use (either alone or in combination with other active ingredients), the use for which it was approved, and the provision of law under which it was approved.**

As active ingredients, a single dose of the approved product contains approximately 20 mcg of HPV 6 L1 protein, 40 mcg of HPV 11 L1 protein, 40 mcg of HPV 16 L1 protein, and 20 mcg of HPV 18 L1 protein. The L1 proteins are produced by separate fermentations in recombinant *Saccharomyces cerevisiae* and self-assembled into VLPs.

Neither Gardasil® nor any of the individual active ingredients have been previously approved for commercial marketing or use under the Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act.

(5) **A statement that the application is being submitted within the sixty day period permitted for submission pursuant to § 1.720(f) and an identification of the date of the last day on which the application could be submitted;**

Gardasil® was approved on June 8, 2006, and the last day within the sixty day period permitted for submission of an application for patent term extension is August 8, 2006, which is subsequent to the date on which this application has been submitted.

(6) **A complete identification of the patent for which an extension is being sought by the name of the inventor, the patent number, the date of issue, and the date of expiration;**

A complete identification of the patent for which an extension is being sought is as follows:

Inventors:	Joseph G. Joyce
	Hugh A. George
	Kathryn J. Hofmann
	Kathrin U. Jansen
	Michael P. Neeper
U.S. Patent No.:	5,820,870
Earliest Filing Date:	March 22, 1995
Grant Date:	October 13, 1998
Expiration Date	October 13, 2015

(7) A copy of the patent for which an extension is being sought, including the entire specification (including claims) and drawings;

A full copy of U.S. Patent No. 5,820,870, for which extension is being sought, is attached as **Exhibit 4**.

(8) A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment, or reexamination certificate issued in the patent;

No Certificate of Correction has been filed and/or issued for U.S. Patent No. 5,820,870.

A copy of the maintenance fee statements, which evidence timely payment of each maintenance fee when due, is attached as **Exhibit 5**.

No disclaimer or reexamination certificate has been filed and/or issued for U.S. Patent No. 5,820,870.

(9) A statement that the patent claims the approved product, or a method of using or manufacturing the approved product, and a showing which lists each applicable patent claim and demonstrates the manner in which at least one such patent claim reads on:

Claims of U.S. Patent No. 5,820,870 read on the approved product as detailed below.

As described on page 1 of the Approved Label (Exhibit 2), Gardasil® is a non-infectious, recombinant, quadrivalent Human Papillomavirus vaccine that contains highly purified VLPs of the major capsid (L1) protein of HPV Types 6, 11, 16, and 18. The L1 proteins are produced by separate fermentations in recombinant *Saccharomyces cerevisiae* and self-assembled into VLPs. The fermentation process involves growth of *S. cerevisiae* on chemically-

defined fermentation media which includes vitamins, amino acids, mineral salts, and carbohydrates. The VLPs are released from the yeast cells by cell disruption and purified by a series of chemical and physical methods. The purified VLPs are adsorbed on preformed aluminum-containing adjuvant (amorphous aluminum hydroxyphosphate sulfate). The quadrivalent HPV VLP vaccine is a sterile liquid suspension that is prepared by combining the adsorbed VLPs of each HPV type (6, 11, 16, and 18) and additional amounts of the aluminum-containing adjuvant and the final purification buffer.

U.S. Patent 5,820,870 contains claims to purified HPV 18 L1 VLPs comprising recombinant HPV 18 L1 protein having a defined amino acid sequence (SEQ ID NO:2). The specifically defined HPV 18 VLPs are a component of Gardasil®, as evidenced by the inclusion of this defined HPV 18 L1 amino acid sequence in BB IND 9030 (see pages 20-21, Figure 7.9 of BB IND 9030, set forth herein as **Exhibit 6**).

The following is a demonstration of the manner in which specific claims of the '870 patent read on:

(i) The approved product, if the listed claims include any claim to the approved product;

Claim	Demonstration
1. Isolated or purified virus-like particles comprising recombinant Human Papillomavirus type 18 L1 protein having the amino acid sequence of SEQ ID No:2.	Gardasil® is a quadrivalent vaccine comprising HPV VLPs of types 6, 11, 16, and 18. The HPV 18 VLP component of Gardasil® comprises HPV 18 L1 protein having the amino acid sequence shown in SEQ ID NO:2 of the '870 patent.
3. The virus-like particles of claim 1, wherein said particles are produced by expression of a recombinant nucleic acid encoding SEQ ID NO:2.	The HPV 18 VLPs in Gardasil® are produced by expression of a recombinant nucleic acid encoding SEQ ID NO:2.
5. A vaccine comprising a pharmaceutically acceptable carrier and an immunoprotective amount of the virus-like particles of claim 1.	Gardasil® comprises (1) an immunoprotective amount of purified VLPs comprising HPV 18 L1 protein having the amino acid of SEQ ID NO:2 of the '870 patent and (2) a pharmaceutically acceptable carrier.

(ii) The method of using the approved product, if the listed claims include any claim to the method of using the approved product; and

7. A method of preventing papillomavirus infection in a host comprising administering the vaccine of claim 5 to a host.	Gardasil® is a vaccine comprising (1) an immunoprotective amount of purified VLPs comprising HPV 18 L1 protein having the amino acid of SEQ ID NO:2 of the '870 patent and (2) a pharmaceutically acceptable carrier. Gardasil® is used to prevent papillomavirus infection in a host by its administration to said host.
10. A method of inducing an immune response in an animal comprising administering the virus-like particle claim 1 to the animal.	Gardasil is used to induce an immune response in a human to HPV 6, 11, 16, and 18. Gardasil® comprises HPV 18 L1 protein having the amino acid sequence shown in SEQ ID NO:2 of the '870 patent. Administration of Gardasil to a human induces an immune response in the human.

(iii) The method of manufacturing the approved product, if the listed claims include any claim to the method of manufacturing the approved product;

No claims of U.S. Patent No. 5,820,870 are directed toward a method of manufacturing the approved product.

(10) A statement beginning on a new page of the relevant dates and information pursuant to 35 U.S.C. 156(g) in order to enable the Secretary of Health and Human Services or the Secretary of Agriculture, as appropriate, to determine the applicable regulatory review period as follows:

(i) For a patent claiming a human drug, antibiotic, or human biological product:

(A) The effective date of the investigational new drug (IND) application and the IND number;

The first IND application for the approved product was submitted to the FDA by Merck on April 14, 2000. By letter dated April 19, 2000, the FDA acknowledged receipt of the IND application on April 14, 2000, and assigned IND number BB-IND 9030, resulting in an IND effective date of **May 14, 2000**. A copy of the FDA acknowledgement letter is attached as

Exhibit 7. The title of BB-IND 9030 is "Human Papillomavirus Quadrivalent (Types 6, 11, 16 and 18; S. cerevisiae) L1 Capsid Virus Like Particle Vaccine with Alum."

Under these circumstances, the "regulatory review period" under 35 U.S.C. § 156(g)(1) began on **May 14, 2000**, the effective date of BB-IND 9030.

(B) The date on which a new drug application (NDA) or a Product License Application (PLA) was initially submitted and the NDA or PLA number; and

The BLA for Gardasil® was initially submitted to the FDA on December 1, 2005. By letter dated December 12, 2005, the FDA acknowledged receipt of the BLA on December 7, 2005, and assigned Submission Tracking Number (STN): BL 125126, as confirmed by **Exhibit 8**. This establishes **December 7, 2005** as the initial submission date of the BLA for the approved product for purposes of 35 U.S.C. § 156(g)(1).

(C) The date on which the NDA was approved or the Product License issued;

The BLA was approved by the FDA approval letter dated and sent June 8, 2006, setting the effective date of the approval as the June 8, 2006 date of the letter. A copy of this FDA approval letter is attached as **Exhibit 3**. This establishes the end of the "regulatory review period" under 35 U.S.C. 156(g)(1) as **June 8, 2006**.

(11) A brief description beginning on a new page of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities;

A listing of the more significant activities undertaken by the marketing applicant with respect to the approved product during the applicable regulatory review period is attached as **Exhibit 9**, the disclosure of which is incorporated herein in its entirety.

(12) A statement beginning on a new page that in the opinion of the applicant the patent is eligible for the extension and a statement as to the length of extension claimed, including how the length of extension was determined;

Statement That the Patent Is Eligible For Extension

Applicant is of the opinion that U.S. Patent No. 5,820,870 is eligible for extension under 35 U.S.C. § 156(a) because it satisfies all of the requirements for such extension as follows:

(1) 35 U.S.C. 156(a)

U.S. Patent No. 5,820,870 claims the approved product as detailed in section (9) above.

(2) 35 U.S.C. 156(a)(1)

U.S. Patent No. 5,820,870 was granted on October 13, 1998 on an earliest filed U.S. application filed on March 22, 1995 and there were no terminal disclaimers. As such, the patent expires on October 13, 1998, being 17 years from grant. This application, therefore, has been submitted before the expiration of the patent term.

(3) 35 U.S.C. 156(a)(2)

The term of this patent has never been extended.

(4) 35 U.S.C. 156(a)(3)

This application is being submitted by the owners of record of U.S. Patent No. 5,820,870 through an assignment from the inventors as detailed on pages 1-2 above and in **Exhibit 1**, in accordance with the requirement of 35 U.S.C. 156(d) and rules of the U.S. Patent and Trademark Office.

(5) 35 U.S.C. 156(a)(4)

As evidenced by the June 8, 2006 approval letter from the FDA (Exhibit 3), Gardasil® was subject to a regulatory review period under Section 351 of the Public Health Service Act (42 U.S.C. § 262) before its commercial marketing or use.

(6) 35 U.S.C. 156(a)(5)(A)

The permission for commercial marketing of Gardasil® after this regulatory review period is the first permitted commercial marketing of the approved product or any active ingredient thereof, under provision of the Public Health Service Act (42 U.S.C. § 262) under which the regulatory review period occurred, as confirmed by the absence of any approved BLA for the approved product or any active ingredient thereof prior to June 8, 2006.

(7) 35 U.S.C. 156(a)(5)(B)

No other patent has been extended for the same regulatory review period for the product Gardasil®.

Statement Regarding Length of Extension Claimed

The term of U.S. Patent 5,820,870 should be extended 1200 days, from October 13, 2015 to January 25, 2019. In accordance with the implementing regulations of 37 C.F.R. 1.775 with respect to patent term extensions for a human drug product, the term extension of U.S. Patent No. 5,820,870 based on the regulatory review of Gardasil® was determined as follows:

Sec. 1.775 Calculation of patent term extension for a human drug, antibiotic drug or human biological product.

(a) If a determination is made pursuant to § 1.750 that a patent for a human drug, antibiotic drug or human biological product is eligible for extension, the term shall be extended by the time as calculated in days in the manner indicated by this section. The patent term extension will run from the original expiration date of the patent or any earlier date set by terminal disclaimer (§ 1.321).

U.S. Patent 5,820,870 issued on October 13, 1998 from an earliest filed U.S. application filed on March 22, 1995. Pursuant to 35 U.S.C. 154(c), this patent is entitled to an original term of 17 years from its grant on October 13, 1998, which provides an original expiration date of October 13, 2015.

(b) The term of the patent for a human drug, antibiotic drug or human biological product will be extended by the length of the regulatory review

period for the product as determined by the Secretary of Health and Human Services, reduced as appropriate pursuant to paragraphs (d)(1) through (d)(6) of this section.

(c) The length of the regulatory review period for a human drug, antibiotic drug or human biological product will be determined by the Secretary of Health and Human Services. Under 35 U.S.C. 156(g)(1)(B), it is the sum of--

(1) The number of days in the period beginning on the date an exemption under subsection (i) of section 505 or subsection (d) of section 507 of the Federal Food, Drug, and Cosmetic Act became effective for the approved product and ending on the date the application was initially submitted for such product under those sections or under section 351 of the Public Health Service Act; and

(2) The number of days in the period beginning on the date the application was initially submitted for the approved product under section 351 of the Public Health Service Act, subsection (b) of section 505 or section 507 of the Federal Food, Drug, and Cosmetic Act and ending on the date such application was approved under such section.

The number of days in the IND testing period of paragraph (c)(1) extends from the effective date of BB-IND 9030 on May 14, 2000 to the filing (receipt) of STN:BL 125126 on December 7, 2005, being **2033 days**.

The number of days in the NDA approval period of paragraph (c)(2) extends from the filing of STN:BL 125126 on December 7, 2005 to the date of approval of STN:BL 125126 on June 8, 2006, being **183 days**.

The regulatory review period is the sum of the periods of paragraphs (c)(1) and (c)(2), being **2216 days**.

(d) The term of the patent as extended for a human drug, antibiotic drug or human biological product will be determined by--

(1) Subtracting from the number of days determined by the Secretary of Health and Human Services to be in the regulatory review period:

(i) The number of days in the periods of paragraphs (c)(1) and (c)(2) of this section which were on and before the date on which the patent issued;

(ii) The number of days in the periods of paragraphs (c)(1) and (c)(2) of this section during which it is determined under 35 U.S.C. 156(d)(2)(B) by the Secretary of Health and Human Services that applicant did not act with due diligence;

(iii) One-half the number of days remaining in the period defined by paragraph (c)(1) of this section after that period is reduced in accordance with paragraphs (d)(1)(i) and (ii) of this section; half days will be ignored for purposes of subtraction;

With respect to paragraph (d)(1)(i), **0 (zero) days** of the periods of paragraphs (c)(1) and (c)(2) were before the October 13, 1998 date on which U.S. Patent No. 5,820,870 issued.

With respect to paragraph (d)(1)(ii), 35 U.S.C. 156 (d)(2)(B) provides that if a petition is submitted to the Secretary not later than 180 days after publication of the determination of the applicable regulatory review period, upon which it may reasonably be determined that the applicant did not act with due diligence during the applicable regulatory review period, the Secretary shall determine if the applicant acted with due diligence during the applicable regulatory review period. The Secretary making this determination shall notify the Director of the determination and shall publish in the Federal register a notice of such determination together with the factual and legal basis for such determination. Any interested person may request, within the 60-day period beginning on the publication of a determination, the Secretary to hold an informal hearing on the determination. If such request is made within such period, the Secretary shall hold such hearing, and shall provide notice of the hearing to the owner of the patent involved and to any interested person and provide the owner and any interested person an opportunity to participate in the hearing. Within 30 days after the completion of the hearing, the secretary shall affirm or revise the determination which was the subject of the hearing and shall notify the Director of any revision of the determination and shall publish any such revision in the Federal Register. There has been no such petition or determination by the Secretary, and thus the number of days under (d)(1)(ii) is **0 (zero) days.**

With respect to paragraph (d)(1)(iii), one-half of the number of days remaining in the period defined by paragraph (c)(1) after that period is reduced in accordance with paragraphs (d)(1)(i) and (ii) is one-half of (2033-0-0) days, which is **1016 days** (ignoring the half-day).

Subtracting from the regulatory review period of 2216 days as determined above pursuant to section 1.775(c) the number of days determined above with respect to paragraphs

(d)(1)(i), (ii) and (iii), the term of patent extension is **2216 days** minus **0 (zero) days** minus **0 (zero) days** minus **1016 days** for a sum total of **1200 days**.

(2) By adding the number of days determined in paragraph (d)(1) of this section to the original term of the patent as shortened by any terminal disclaimer;

The original term of U.S. Patent No. 5,820,870 is October 13, 2015 and is not shortened by terminal disclaimer. Adding the 1200 days determined in paragraph (d)(1) to the original term of the patent results in an extended term to **January 25, 2019**.

(3) By adding 14 years to the date of approval of the application under section 351 of the Public Health Service Act, or subsection (b) of section 505 or section 507 of the Federal Food, Drug, and Cosmetic Act;

Adding 14 years to the June 8, 2006 date of approval of the BLA results in the date **June 8, 2020**.

(4) By comparing the dates for the ends of the periods obtained pursuant to paragraphs (d)(2) and (d)(3) of this section with each other and selecting the earlier date;

The earlier of January 25, 2019 and June 8, 2020 is **January 25, 2019**.

(5) If the original patent was issued after September 24, 1984,

(i) By adding 5 years to the original expiration date of the patent or any earlier date set by terminal disclaimer; and

(ii) By comparing the dates obtained pursuant to paragraphs (d)(4) and (d)(5)(i) of this section with each other and selecting the earlier date;

The original patent issued after September 24, 1984. Adding 5 years to the original expiration date of the patent (there was no terminal disclaimer) of October 13, 2015 gives a date of **October 13, 2020**. The earlier of January 25, 2019 and October 13, 2020 is **January 25, 2019**.

(6) If the original patent was issued before September 24, 1984, and

(i) If no request was submitted for an exemption under subsection (i) of section 505 or subsection (d) of section 507 of the Federal Food, Drug, and Cosmetic Act before September 24, 1984, by--

(A) Adding 5 years to the original expiration date of the patent or earlier date set by terminal disclaimer; and

(B) By comparing the dates obtained pursuant to paragraphs (d)(4) and (d)(6)(i)(A) of this section with each other and selecting the earlier date; or

(ii) If a request was submitted for an exemption under subsection (i) of section 505 or subsection (d) of section 507 of the Federal Food, Drug, or Cosmetic Act before September 24, 1984 and the commercial marketing or use of the product was not approved before September 24, 1984, by--

(A) Adding 2 years to the original expiration date of the patent or earlier date set by terminal disclaimer, and

(B) By comparing the dates obtained pursuant to paragraphs (d)(4) and (d)(6)(ii)(A) of this section with each other and selecting the earlier date.

Since U.S. Patent 5,820,870 issued after September 24, 1984, no further adjustments to the extended term of January 25, 2019 is required.

Thus, as calculated above, the term of U.S. Patent No. 5,820,870 is eligible for a **1200 day extension to January 25, 2019**.

(13) A statement that applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought (see § 1.765);

Applicant acknowledges a duty to disclose to the Patent and Trademark Office and the Secretary of Health and Human Services any information which is material to any determination of entitlement to the extension sought.

(14) The prescribed fee for receiving and acting upon the application for extension (see § 1.20(j)); and

As noted in the letter of transmittal submitted with this application, the Patent and Trademark Office is authorized to charge the filing fee of $1,120.00 and any additional fees

which may be required by this or any other related paper, or to credit any overpayment to Deposit Account No. 13-2755.

(15) The name, address, and telephone number of the person to whom inquiries and correspondence relating to the application for patent term extension are to be directed.

Please address all inquiries and correspondence relating to this application for patent term extension to the following registered practitioner who has been authorized by the assignee to act on its behalf with respect to the filing of this application and all correspondence pertaining thereto:

Joanne M. Giesser
Registration Number 32,838
MERCK & CO., INC.
P.O. Box 2000
Rahway, New Jersey 07065-0907
Tel. No.: (732) 594-3046
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Respectfully submitted,

By Joanne M. Giesser

Joanne M. Giesser
Reg. No. 32,838
Attorney for Applicant

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Date: August 2, 2006

EXHIBIT 1
5 PAGES

EXHIBIT 1
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UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
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OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

MARCH 05, 1998

PTAS

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JOANNE M. GIESSER
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RAHWAY, NJ 07065-0907

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, NORTH TOWER BUILDING, SUITE 10C35, WASHINGTON, D.C. 20231.

RECORDATION DATE: 01/20/1998
REEL/FRAME: 8908/0030
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
JOYCE, JOSEPH G. DOC DATE: 03/21/1995

ASSIGNOR:
GEORGE, HUGH A. DOC DATE: 03/21/1995

ASSIGNOR:
HOFMANN, KATHRYN J. DOC DATE: 03/21/1995

ASSIGNOR:
JANSEN, KATHRIN U. DOC DATE: 03/21/1995

ASSIGNOR:
NEEPER, MICHAEL P. DOC DATE: 03/21/1995

ASSIGNEE:
MERCK & CO., INC.
P.O. BOX 2000
RAHWAY, NEW JERSEY 07065-0907

SERIAL NUMBER: 08409122
PATENT NUMBER:

FILING DATE: 03/22/1995
ISSUE DATE:

SEDLEY PYNE, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

Patent Case No. 19425

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

01-29-1998

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TRADEMARKS:
OR COPY THEREOF.

1. Name(s) of Conveying party(ies):
JOSEPH G. JOYCE, HUGH A. GEORGE, KATHRYN J. HOFMANN, KATHRIN U. JANSEN, MICHAEL P. NEEPER

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

2. Nature of conveyance:
- ☒ Assignment
- ☐ Merger
- ☐ Security Agreement
- ☐ Change of Name
- ☐ Other:

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date appearing below.

MERCK & CO., INC.

By Joanne M. Giesser Date 1/13/98

3. Name and address of receiving party(ies):
Name: MERCK & CO., INC.
Internal Address: RY60-30
Street Address: P.O. Box 2000
City & State: RAHWAY, NEW JERSEY
Zip: 07065-0907

Execution Date: Mar 21, 1995

Additional name(s) & addresses attached? ☐ Yes ☒ No

4. Application number(s) or patent number(s) are as follows:
(a) Patent Application No(s). 08/409,122, filed on Mar 22, 1995, and titled:
RECOMBINANT HUMAN PAPILLOMAVIRUS TYPE 18 VACCINE

(b) If this document is being filed together with a new application, the execution date of the application:

Additional numbers attached? ☐ Yes ☒ No

5. Name & address of party to whom correspondence concerning documents should be mailed:
Name: JOANNE M. GIESSER
Internal Address: PATENT DEPARTMENT
MERCK & CO., INC.
P.O. BOX 2000 -- RY60-30
City & State: RAHWAY, N.J. Zip: 07065-0907

6. Total no. of applications & patents involved: 1

7. Total fee (37 CFR 3.41).....$ 40.00
☐ Enclosed
☒ The Commissioner is hereby authorized to charge deposit account number 13-2755 for any fees which may be required or to credit any overpayment.

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8. Statement and signature:
To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

JOANNE M. GIESSER Reg. No. 32,838 Joanne M. Giesser 1/13/98
Name of Person Signing Signature Date

Total number of pages including cover sheet, attachments, and document: 3

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Rev. 8/5/93

PATENT
JOINT Merck Case 19425
U.S. Serial No. 08/409,122
Filing Date March 22, 1995

ASSIGNMENT AND AGREEMENT

For value received, we, JOSEPH G. JOYCE, HUGH A. GEORGE, KATHRYN J. HOFMANN, KATHRIN U. JANSEN AND MICHAEL P. NEEPER

of 14 GLEN ROAD, LANSDALE, PA 19446, 124 SPRING MT. ROAD, SCHWENKSVILLE, PA 19473, 1845 MORGAN LANE, COLLEGEVILLE, PA 19426, 427 LEAH DRIVE, FT. WASHINGTON, PA 19034, AND 1845 MORGAN LANE, COLLEGEVILLE, PA 19426

hereby sell, assign and transfer to MERCK & CO., Inc., a corporation of the State of New Jersey, having an office at Lincoln Avenue, City of Rahway, State of New Jersey, and it successors, assigns and legal representatives, the entire right, title and interest, for all countries, in and to certain inventions relating to

RECOMBINANT HUMAN PAPILLOMAVIRUS TYPE 18 VACCINE

described in an application for Letters Patent of the United States, executed by each of us on even date herewith, or executed on the date shown in the Declaration and Power of Attorney relating to said application, and all the rights and privileges, including any and all benefits under the International Convention for the Protection of Industrial Property and related treaties, under any and all Letters Patents which may be granted in any foreign country, and under any and all extensions, divisionals, reissues and continuations of said Letters Patents.

We request that any and all Patents for said inventions be issued to said assignee, its successor, assigns and legal representatives, or to such nominees as it may designate.

We agree that, when requested, we will, without charge to said assignee but at its expense, sign all papers, take all rightful oaths, and do all acts which may be necessary, desirable or convenient for securing and maintaining Patents for said inventions in any and all countries and for vesting title thereto in said assignee, its successors, assigns and legal representatives or nominees.

We covenant with said assignee, its successors, assigns and legal representatives, that the rights and property herein conveyed are free and clear of any encumbrance, and that we have full right to convey the same as herein expressed.

We hereby authorize our attorney, CHRISTINE E. CARTY or an attorney with Power of Attorney in this application, of the said MERCK & CO., Inc., to insert **Serial No.**, and **Filing Date** of said application(s) when known.

Signed at WEST POINT, PA this 21ST day of MARCH, 1995

Joseph G. Joyce

Kathrin U. Jansen

Hugh A. George

Michael P. Neeper

Kathryn J. Hofmann

PATENT
JOINT Merck Case 19425
U.S. Serial No. 08/409,122
Filing Date March 22, 1995

ASSIGNMENT AND AGREEMENT

Signed at _____ this _____ day of _____.

_____

_____

_____

_____

COMMONWEALTH OF PENNSYLVANIA
County of MONTGOMERY } SS.

Personally appeared before me the above-named JOSEPH G. JOYCE HUGH A. GEORGE KATHRYN J. HOFMANN KATHRIN U. JANSEN MICHAEL P. NEEPER

to me known and known to me to be the person(s) who executed the foregoing instrument and acknowledged said instrument to be their free act and deed this 21ST day of MARCH, 1995.

Brenda Gallew
Notary Public

Notarial Seal
Brenda Gallew, Notary Public
Upper Gwynedd Twp., Montgomery County
My Commission Expires April 11, 1998
Member, Pennsylvania Association of Notaries

_____
County of _____ } SS.

Personally appeared before me the above-named

to me known and known to me to be the person(s) who executed the foregoing instrument and acknowledged said instrument to be their free act and deed this _____ day of _____.

_____
Notary Public

EXHIBIT 2
18 PAGES

MERCK & CO., INC.
Whitehouse Station, NJ 08889, USA

GARDASIL®

[Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine]

DESCRIPTION

GARDASIL* is a non-infectious recombinant, quadrivalent vaccine prepared from the highly purified virus-like particles (VLPs) of the major capsid (L1) protein of HPV Types 6, 11, 16, and 18. The L1 proteins are produced by separate fermentations in recombinant *Saccharomyces cerevisiae* and self-assembled into VLPs. The fermentation process involves growth of *S. cerevisiae* on chemically-defined fermentation media which include vitamins, amino acids, mineral salts, and carbohydrates. The VLPs are released from the yeast cells by cell disruption and purified by a series of chemical and physical methods. The purified VLPs are adsorbed on preformed aluminum-containing adjuvant (amorphous aluminum hydroxyphosphate sulfate). The quadrivalent HPV VLP vaccine is a sterile liquid suspension that is prepared by combining the adsorbed VLPs of each HPV type and additional amounts of the aluminum-containing adjuvant and the final purification buffer.

GARDASIL is a sterile preparation for intramuscular administration. Each 0.5-mL dose contains approximately 20 mcg of HPV 6 L1 protein, 40 mcg of HPV 11 L1 protein, 40 mcg of HPV 16 L1 protein, and 20 mcg of HPV 18 L1 protein.

Each 0.5-mL dose of the vaccine contains approximately 225 mcg of aluminum (as amorphous aluminum hydroxyphosphate sulfate adjuvant), 9.56 mg of sodium chloride, 0.78 mg of L-histidine, 50 mcg of polysorbate 80, 35 mcg of sodium borate, and water for injection. The product does not contain a preservative or antibiotics.

After thorough agitation, GARDASIL is a white, cloudy liquid.

CLINICAL PHARMACOLOGY

Disease Burden

Human Papillomavirus (HPV) causes squamous cell cervical cancer (and its histologic precursor lesions Cervical Intraepithelial Neoplasia [CIN] 1 or low grade dysplasia and CIN 2/3 or moderate to high grade dysplasia) and cervical adenocarcinoma (and its precursor lesion adenocarcinoma *in situ* [AIS]). HPV also causes approximately 35-50% of vulvar and vaginal cancers. Vulvar Intraepithelial Neoplasia (VIN) Grade 2/3 and Vaginal Intraepithelial Neoplasia (VaIN) Grade 2/3 are immediate precursors to these cancers.

Cervical cancer prevention focuses on routine screening and early intervention. This strategy has reduced cervical cancer rates by approximately 75% in compliant individuals by monitoring and removing premalignant dysplastic lesions.

HPV also causes genital warts (condyloma acuminata) which are growths of the cervicovaginal, vulvar, and the external genitalia that rarely progress to cancer. HPV 6, 11, 16, and 18 are common HPV types.

HPV 16 and 18 cause approximately:

- 70% of cervical cancer, AIS, CIN 3, VIN 2/3, and VaIN 2/3 cases; and
- 50% of CIN 2 cases.

HPV 6, 11, 16, and 18 cause approximately:

- 35 to 50% of all CIN 1, VIN 1, and VaIN 1 cases; and
- 90% of genital wart cases.

Mechanism of Action

HPV only infects humans, but animal studies with analogous (animal, not human) papillomaviruses suggest that the efficacy of L1 VLP vaccines is mediated by the development of humoral immune responses.

CLINICAL STUDIES

CIN 2/3 and AIS are the immediate and necessary precursors of squamous cell carcinoma and adenocarcinoma of the cervix, respectively. Their detection and removal has been shown to prevent cancer; thus, they serve as surrogate markers for prevention of cervical cancer.

Efficacy was assessed in 4 placebo-controlled, double-blind, randomized Phase II and III clinical studies. The first Phase II study evaluated the HPV 16 component of GARDASIL (Protocol 005, N = 2391) and the second evaluated all components of GARDASIL (Protocol 007, N = 551). The Phase III studies, termed FUTURE (Females United To Unilaterally Reduce Endo/Ectocervical Disease), evaluated GARDASIL in 5442 (FUTURE I or Protocol 013) and 12,157 (FUTURE II or Protocol 015) subjects. Together, these four studies evaluated 20,541 women 16 to 26 years of age at enrollment. The median duration of follow-up was 4.0, 3.0, 2.4, and 2.0 years for Protocol 005, Protocol 007, FUTURE I, and FUTURE II, respectively. Subjects received vaccine or placebo on the day of enrollment, and 2 and 6 months thereafter. Efficacy was analyzed for each study individually and for all studies combined according to a prospective clinical plan.

Prophylactic Efficacy

GARDASIL is designed to prevent HPV 6-, 11-, 16-, and/or 18-related cervical cancer, cervical dysplasias, vulvar or vaginal dysplasias, or genital warts. GARDASIL was administered without prescreening for presence of HPV infection and the efficacy trials allowed enrollment of subjects regardless of baseline HPV status (i.e., Polymerase Chain Reaction [PCR] status or serostatus). Subjects who were infected with a particular vaccine HPV type (and who may already have had disease due to that infection) were not eligible for prophylactic efficacy evaluations for that type.

The primary analyses of efficacy were conducted in the per-protocol efficacy (PPE) population, consisting of individuals who received all 3 vaccinations within 1 year of enrollment, did not have major deviations from the study protocol, and were naïve (PCR negative in cervicovaginal specimens and seronegative) to the relevant HPV type(s) (Types 6, 11, 16, and 18) prior to dose 1 and through 1 month Postdose 3 (Month 7). Efficacy was measured starting after the Month 7 visit.

Overall, 73% of subjects were naïve (i.e., PCR negative and seronegative for all 4 vaccine HPV types) to all 4 vaccine HPV types at enrollment.

A total of 27% of subjects had evidence of prior exposure to or ongoing infection with at least 1 of the 4 vaccine HPV types. Among these subjects, 74% had evidence of prior exposure to or ongoing infection with only 1 of the 4 vaccine HPV types and were naïve (PCR negative and seronegative) to the remaining 3 types.

In subjects who were naïve (PCR negative and seronegative) to all 4 vaccine HPV types, CIN, genital warts, VIN, and VaIN caused by any of the 4 vaccine HPV types were counted as endpoints.

Among subjects who were positive (PCR positive and/or seropositive) for a vaccine HPV type at Day 1, endpoints related to that type were not included in the analyses of prophylactic efficacy. Endpoints related to the remaining types for which the subject was naïve (PCR negative and seronegative) were counted.

For example, in subjects who were HPV 18 positive (PCR positive and/or seropositive) at Day 1, lesions caused by HPV 18 were not counted in the prophylactic efficacy evaluations. Lesions caused by HPV 6, 11, and 16 were included in the prophylactic efficacy evaluations. The same approach was used for the other types.

GARDASIL was efficacious in reducing the incidence of CIN (any grade including CIN 2/3); AIS; genital warts; VIN (any grade); and VaIN (any grade) related to vaccine HPV types in those who were PCR negative and seronegative at baseline (Table 1).

Table 1
Analysis of Efficacy of GARDASIL in the PPE* Population**

Population	GARDASIL		Placebo		% Efficacy (95% CI)
	n	Number of cases	n	Number of cases	
HPV 16- or 18-related CIN 2/3 or AIS					
Protocol 005***	755	0	750	12	100.0 (65.1, 100.0)
Protocol 007	231	0	230	1	100.0 (-3734.9, 100.0)
FUTURE I	2200	0	2222	19	100.0 (78.5, 100.0)
FUTURE II	5301	0	5258	21	100.0† (80.9, 100.0)
Combined Protocols‡	8487	0	8460	53	100.0† (92.9, 100.0)
HPV 6-, 11-, 16-, 18-related CIN (CIN 1, CIN 2/3) or AIS					
Protocol 007	235	0	233	3	100.0 (-137.8, 100.0)
FUTURE I	2240	0	2258	37	100.0† (89.5, 100.0)
FUTURE II	5383	4	5370	43	90.7 (74.4, 97.6)
Combined Protocols	7858	4	7861	83	95.2 (87.2, 98.7)
HPV 6-, 11-, 16-, or 18-related Genital Warts					
Protocol 007	235	0	233	3	100.0 (-139.5, 100.0)
FUTURE I	2261	0	2279	29	100.0 (86.4, 100.0)
FUTURE II	5401	1	5387	59	98.3 (90.2, 100.0)
Combined Protocols	7897	1	7899	91	98.9 (93.7, 100.0)

*The PPE population consisted of individuals who received all 3 vaccinations within 1 year of enrollment, did not have major deviations from the study protocol, and were naïve (PCR negative and seronegative) to the relevant HPV type(s) (Types 6, 11, 16, and 18) prior to dose 1 and through 1 month Postdose 3 (Month 7).
**See Table 2 for analysis of vaccine impact in the general population.
***Evaluated only the HPV 16 L1 VLP vaccine component of GARDASIL.
†P-values were computed for pre-specified primary hypothesis tests. All p-values were <0.001, supporting the following conclusions: efficacy against HPV 16/18-related CIN 2/3 is >0% (FUTURE II); efficacy against HPV 16/18-related CIN 2/3 is >25% (Combined Protocols); and efficacy against HPV 6/11/16/18-related CIN is >20% (FUTURE I).
‡Analyses of the combined trials were prospectively planned and included the use of similar study entry criteria.
n = Number of subjects with at least 1 follow-up visit after Month 7.
Note 1: Point estimates and confidence intervals are adjusted for person-time of follow-up.
Note 2: The first analysis in the table (i.e., HPV 16- or 18-related CIN 2/3, AIS or worse) was the primary endpoint of the vaccine development plan.
Note 3: FUTURE I refers to Protocol 013; FUTURE II refers to Protocol 015.

GARDASIL was efficacious against HPV disease caused by each of the 4 vaccine HPV types.

In a pre-defined analysis, the efficacy of GARDASIL against HPV 16/18-related disease was 100% (95% CI: 87.9%, 100.0%) for CIN 3 or AIS and 100% (95% CI: 55.5%, 100.0%) for VIN 2/3 or VaIN 2/3. The efficacy of GARDASIL against HPV 6-, 11-, 16-, and 18-related VIN 1 or VaIN 1 was 100% (95% CI: 75.8%, 100.0%). These analyses were conducted in the PPE population that consisted of individuals who received all 3 vaccinations within 1 year of enrollment, did not have major deviations from the study protocol, and were naïve (PCR negative and seronegative) to the relevant HPV type(s) (Types 6, 11, 16, and 18) prior to dose 1 and through 1 month Postdose 3 (Month 7).

Efficacy in Subjects with Current or Prior Infection

GARDASIL is a prophylactic vaccine.

There was no clear evidence of protection from disease caused by HPV types for which subjects were PCR positive and/or seropositive at baseline.

Individuals who were already infected with 1 or more vaccine-related HPV types prior to vaccination were protected from clinical disease caused by the remaining vaccine HPV types.

General Population Impact

The general population of young American women includes women who are HPV-naïve (PCR negative and seronegative) and women who are HPV-non-naïve (PCR positive and/or seropositive), some of whom have HPV-related disease. The clinical trials population approximated the general population of American women with respect to prevalence of HPV infection and disease at enrollment. Analyses were conducted to evaluate the overall impact of GARDASIL with respect to HPV 6-, 11-, 16-, and 18-related cervical and genital disease in the general population. Here, analyses included events arising from HPV infections that were present at the start of vaccination as well as events that arose from infections that were acquired after the start of vaccination.

The impact of GARDASIL in the general population is shown in Table 2. Impact was measured starting 1 month Postdose 1. Prophylactic efficacy denotes the vaccine's efficacy in women who are naïve (PCR negative and seronegative) to the relevant HPV types at vaccination onset. General population impact denotes vaccine impact among women regardless of baseline PCR status and serostatus. The majority of CIN and genital warts, VIN, and VaIN detected in the group that received GARDASIL occurred as a consequence of HPV infection with the relevant HPV type that was already present at Day 1.

Table 2
General Population Impact for Vaccine HPV Types

Endpoints	Analysis	GARDASIL or HPV 16 L1 VLP Vaccine		Placebo		% Reduction (95% CI)
		N	Cases	N	Cases	
HPV 16- or 18-related CIN 2/3 or AIS	Prophylactic Efficacy*	9342	1	9400	81	98.8 (92.9, 100.0)
	HPV 16 and/or HPV 18 Positive at Day 1	--	121	--	120	--
	General Population Impact**	9831	122	9896	201	39.0 (23.3, 51.7)
HPV 16- or 18-related VIN 2/3 and VaIN 2/3	Prophylactic Efficacy*	8641	0	8667	24	100.0 (83.3, 100.0)
	HPV 16 and/or HPV 18 Positive at Day 1	--	8	--	2	
	General Population Impact**	8954	8	8962	26	69.1 (29.8, 87.9)
HPV 6-, 11-, 16-, 18-related CIN (CIN 1, CIN 2/3) or AIS	Prophylactic Efficacy*	8625	9	8673	143	93.7 (87.7, 97.2)
	HPV 6, HPV 11, HPV 16, and/or HPV 18 Positive at Day 1	--	161***	--	174***	--
	General Population Impact**	8814	170	8846	317	46.4 (35.2, 55.7)
HPV 6-, 11-, 16-, or 18-related Genital Warts	Prophylactic Efficacy*	8760	9	8786	136	93.4 (87.0, 97.0)
	HPV 6, HPV 11, HPV 16, and/or HPV 18 Positive at Day 1	--	49	--	48†	--
	General Population Impact**	8954	58	8962	184	68.5 (57.5, 77.0)

*Includes all subjects who received at least 1 vaccination and who were naïve (PCR negative and seronegative) to HPV 6, 11, 16, and/or 18 at Day 1. Case counting started at 1 Month Postdose 1.
**Includes all subjects who received at least 1 vaccination (regardless of baseline HPV status at Day 1). Case counting started at 1 Month Postdose 1.
***Includes 2 subjects (1 in each vaccination group) who underwent colposcopy for reasons other than an abnormal Pap and 1 placebo subject with missing serology/PCR data at day 1.
†Includes 1 subject with missing serology/PCR data at day 1.
Note 1: The 16- and 18-related CIN 2/3 or AIS composite endpoint included data from studies 005, 007, 013, and 015. All other endpoints only included data from studies 007, 013, and 015.
Note 2: Positive status at Day 1 denotes PCR positive and/or seropositive for the respective type at Day 1.
Note 3: Percent reduction includes the prophylactic efficacy of GARDASIL as well as the impact of GARDASIL on the course of infections present at the start of the vaccination.
Note 4: Table 2 does not include disease due to non-vaccine HPV types.

GARDASIL does not prevent infection with the HPV types not contained in the vaccine. Cases of disease due to non-vaccine types were observed among recipients of GARDASIL and placebo in Phase II and Phase III efficacy studies.

Among cases of CIN 2/3 or AIS caused by vaccine or non-vaccine HPV types in subjects in the general population who received GARDASIL, 79% occurred in subjects who had an abnormal Pap test at Day 1 and/or who were positive (PCR positive and/or seropositive) to HPV 6, 11, 16, and/or 18 at Day 1.

An interim analysis of the general population impact for GARDASIL was performed from studies 007, 013, and 015 that had a median duration of follow-up of 1.9 years. GARDASIL reduced the overall rate of CIN 2/3 or AIS caused by vaccine or non-vaccine HPV types by 12.2% (95% CI: -3.2%, 25.3%), compared with placebo.

An analysis of overall population impact for the HPV 16 L1 VLP vaccine was conducted from study 005 that had a median duration of follow-up of 3.9 years. The HPV 16 L1 VLP vaccine reduced the overall incidence of CIN 2/3 caused by vaccine or non-vaccine HPV types by 32.7% (95% CI: -34.7%, 67.3%) through a median duration of follow-up of 1.9 years (fixed case analysis) and by 45.3% (95% CI: 10.9%, 67.1%), through a median duration of follow-up of 3.9 years (end of study).

GARDASIL reduced the incidence of definitive therapy (e.g., loop electrosurgical excision procedure, laser conization, cold knife conization) by 16.5% (95% CI: 2.9%, 28.2%), and surgery to excise external

genital lesions by 26.5% (95% CI: 3.6%, 44.2%), compared with placebo for all HPV-related diseases. These analyses were performed in the general population of women which includes women regardless of baseline HPV PCR status or serostatus. GARDASIL has not been shown to protect against the diseases caused by all HPV types and will not treat existing disease caused by the HPV types contained in the vaccine. The overall efficacy of GARDASIL, described above, will depend on the baseline prevalence of HPV infection related to vaccine types in the population vaccinated and the incidence of HPV infection due to types not included in the vaccine.

Immunogenicity

Assays to Measure Immune Response

Because there were few disease cases in subjects naïve (PCR negative and seronegative) to vaccine HPV types at baseline in the group that received GARDASIL, it has not been possible to establish minimum anti-HPV 6, anti-HPV 11, anti-HPV 16, and anti-HPV 18 antibody levels that protect against clinical disease caused by HPV 6, 11, 16, and/or 18.

The immunogenicity of GARDASIL was assessed in 8915 women (GARDASIL N = 4666; placebo N = 4249) 18 to 26 years of age and female adolescents 9 to 17 years of age (GARDASIL N = 1471; placebo N = 583).

Type-specific competitive immunoassays with type-specific standards were used to assess immunogenicity to each vaccine HPV type. These assays measured antibodies against neutralizing epitopes for each HPV type. The scales for these assays are unique to each HPV type; thus, comparisons across types and to other assays are not appropriate.

Immune Response to GARDASIL

The primary immunogenicity analyses were conducted in a per-protocol immunogenicity (PPI) population. This population consisted of individuals who were seronegative and PCR negative to the relevant HPV type(s) at enrollment, remained HPV PCR negative to the relevant HPV type(s) through 1 month Postdose 3 (Month 7), received all 3 vaccinations, and did not deviate from the study protocol in ways that could interfere with the effects of the vaccine.

Overall, 99.8%, 99.8%, 99.8%, and 99.5% of girls and women who received GARDASIL became anti-HPV 6, anti-HPV 11, anti-HPV 16, and anti-HPV 18 seropositive, respectively, by 1 month Postdose 3 across all age groups tested. Anti-HPV 6, anti-HPV 11, anti-HPV 16, and anti-HPV 18 GMTs peaked at Month 7. GMTs declined through Month 24 and then stabilized through Month 36 at levels above baseline (Table 3). The duration of immunity following a complete schedule of immunization with GARDASIL has not been established.

Table 3
Summary of Anti-HPV cLIA Geometric Mean Titers in the PPI* Population

Study Time	GARDASIL N** = 276		Aluminum-Containing Placebo N = 275	
	n***	Geometric Mean Titer (95% CI) mMU/mL†	n	Geometric Mean Titer (95% CI) mMU/mL
Anti-HPV 6				
Month 07	208	582.2 (527.2, 642.8)	198	4.6 (4.3, 4.8)
Month 24	192	93.7 (82.2, 106.9)	188	4.6 (4.3, 5.0)
Month 36	183	93.8 (81.0, 108.6)	184	5.1 (4.7, 5.6)
Anti-HPV 11				
Month 07	208	696.5 (617.8, 785.2)	198	4.1 (4.0, 4.2)
Month 24	190	97.1 (84.2, 112.0)	188	4.2 (4.0, 4.3)
Month 36	174	91.7 (78.3, 107.3)	180	4.4 (4.1, 4.7)
Anti-HPV 16				
Month 07	193	3889.0 (3318.7, 4557.4)	185	6.5 (6.2, 6.9)
Month 24	174	393.0 (335.7, 460.1)	175	6.8 (6.3, 7.4)
Month 36	176	507.3 (434.6, 592.0)	170	7.7 (6.8, 8.8)
Anti-HPV 18				
Month 07	219	801.2 (693.8, 925.4)	209	4.6 (4.3, 5.0)
Month 24	204	59.9 (49.7, 72.2)	199	4.6 (4.3, 5.0)
Month 36	196	59.7 (48.5, 73.5)	193	4.8 (4.4, 5.2)

*The PPI population consisted of individuals who received all 3 vaccinations within pre-defined day ranges, did not have major deviations from the study protocol, met predefined criteria for the interval between the Month 6 and Month 7 visit, and were naïve (PCR negative and seronegative) to the relevant HPV type(s) (Types 6, 11, 16, and 18) prior to dose 1 and through 1 month Postdose 3 (Month 7).
**Number of subjects randomized to the respective vaccination group who received at least 1 injection.
***Number of subjects in the per-protocol analysis with data at the specified study time point.
†mMU = milli-Merck units.
Note: These data are from Protocol 007.

Table 4 compares anti-HPV GMTs 1 month Postdose 3 among subjects who received Dose 2 between Month 1 and Month 3 and subjects who received Dose 3 between Month 4 and Month 8 (Table 4).

Table 4
Summary of GMTs for Variation of Dosing Regimen

Variation of Dosing Regimen	Anti-HPV 6		Anti-HPV 11		Anti-HPV 16		Anti-HPV 18	
	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)
Dose 2								
Early*	883	570.9 (542.2, 601.2)	888	824.6 (776.7, 875.5)	854	2625.3 (2415.1, 2853.9)	926	517.7 (482.9, 555.0)
On Time*	1767	552.3 (532.3, 573.1)	1785	739.7 (709.3, 771.5)	1737	2400.0 (2263.9, 2544.3)	1894	473.9 (451.8, 497.1)
Late*	313	447.4 (405.3, 493.8)	312	613.9 (550.8, 684.2)	285	1889.7 (1624.4, 2198.5)	334	388.5 (348.3, 433.3)
Dose 3								
Early**	495	493.1 (460.8, 527.8)	501	658.9 (609.5, 712.2)	487	2176.6 (1953.4, 2425.3)	521	423.4 (388.8, 461.2)
On Time**	2081	549.6 (531.1, 568.8)	2093	752.8 (723.8, 782.9)	2015	2415.0 (2286.3, 2550.9)	2214	486.0 (464.7, 508.2)
Late**	335	589.0 (537.0, 645.9)	339	865.3 (782.6, 956.7)	326	2765.9 (2408.7, 3176.2)	361	498.5 (446.2, 557.0)

*Early = 36 to 50 days Postdose 1; On-Time = 51 to 70 days Postdose 1; Late = 71 to 84 days Postdose 1.
**Early = 80 to 105 days Postdose 2; On-Time = 106 to 137 days Postdose 2; Late = 138 to 160 days Postdose 2.
Note: GMT = Geometric mean titer in mMU/mL (mMU = milli-Merck units.)

Bridging the Efficacy of GARDASIL from Young Adult Women to Adolescent Girls

A clinical study compared anti-HPV 6, anti-HPV 11, anti-HPV 16, and anti-HPV 18 GMTs in 10- to 15-year-old girls with responses in 16- to 23-year-old adolescent and young adult women. Among subjects who received GARDASIL, 99.1 to 100% became anti-HPV 6, anti-HPV 11, anti-HPV 16, and anti-HPV 18 seropositive by 1 month Postdose 3.

Table 5 compares the 1 month Postdose 3 anti-HPV 6, anti-HPV 11, anti-HPV 16, and anti-HPV 18 GMTs in 9- to 15-year-old girls with those in 16- to 26-year-old adolescent and young adult women.

Table 5
Immunogenicity Bridging Between 9- to 15-year-old Female Adolescents and 16- to 26-year-old Adult Women

Assay (cLIA)	9- to 15-year-old Female Adolescents (Protocols 016 and 018) N = 1121			16- to 26-year-old Adult Women (Protocols 013 and 015) N = 4229		
	n	GMT	(95% CI)	n	GMT	95% CI
Anti-HPV 6	927	931.3	(876.9, 989.2)	2827	542.4	(526.6, 558.7)
Anti-HPV 11	927	1305.7	(1226.2, 1390.4)	2827	766.1	(740.5, 792.6)
Anti-HPV 16	929	4944.9	(4583.5, 5334.8)	2707	2313.8	(2206.2, 2426.7)
Anti-HPV 18	932	1046.0	(971.2, 1126.5)	3040	460.7	(443.8, 478.3)

Note: GMT = Geometric mean titer in mMU/mL (mMU = milli-Merck units).

Anti-HPV responses 1 month Postdose 3 among 9- to 15-year-old girls were non-inferior to anti-HPV responses in 16- to 26-year-old adolescent and young adult women in the combined database of immunogenicity studies for GARDASIL.

On the basis of this immunogenicity bridging, the efficacy of GARDASIL in 9- to 15-year-old girls is inferred.

Studies with Other Vaccines

The safety and immunogenicity of co-administration of GARDASIL with hepatitis B vaccine (recombinant) (same visit, injections at separate sites) were evaluated in a randomized study of 1871 women aged 16 to 24 years at enrollment. Immune response to both hepatitis B vaccine (recombinant) and GARDASIL was non-inferior whether they were administered at the same visit or at a different visit.

INDICATIONS AND USAGE

GARDASIL is a vaccine indicated in girls and women 9-26 years of age for the prevention of the following diseases caused by Human Papillomavirus (HPV) types 6, 11, 16, and 18:

- Cervical cancer
- Genital warts (condyloma acuminata)

and the following precancerous or dysplastic lesions:

- Cervical adenocarcinoma *in situ* (AIS)
- Cervical intraepithelial neoplasia (CIN) grade 2 and grade 3
- Vulvar intraepithelial neoplasia (VIN) grade 2 and grade 3
- Vaginal intraepithelial neoplasia (VaIN) grade 2 and grade 3
- Cervical intraepithelial neoplasia (CIN) grade 1

CONTRAINDICATIONS

Hypersensitivity to the active substances or to any of the excipients of the vaccine.

Individuals who develop symptoms indicative of hypersensitivity after receiving a dose of GARDASIL should not receive further doses of GARDASIL.

PRECAUTIONS

General

As for any vaccine, vaccination with GARDASIL may not result in protection in all vaccine recipients.

This vaccine is not intended to be used for treatment of active genital warts; cervical cancer; CIN, VIN, or VaIN.

This vaccine will not protect against diseases that are not caused by HPV.

GARDASIL has not been shown to protect against diseases due to non-vaccine HPV types.

As with all injectable vaccines, appropriate medical treatment should always be readily available in case of rare anaphylactic reactions following the administration of the vaccine.

The decision to administer or delay vaccination because of a current or recent febrile illness depends largely on the severity of the symptoms and their etiology. Low-grade fever itself and mild upper respiratory infection are not generally contraindications to vaccination.

Individuals with impaired immune responsiveness, whether due to the use of immunosuppressive therapy, a genetic defect, Human Immunodeficiency Virus (HIV) infection, or other causes, may have reduced antibody response to active immunization (see PRECAUTIONS, *Drug Interactions*).

As with other intramuscular injections, GARDASIL should not be given to individuals with bleeding disorders such as hemophilia or thrombocytopenia, or to persons on anticoagulant therapy unless the potential benefits clearly outweigh the risk of administration. If the decision is made to administer GARDASIL to such persons, it should be given with steps to avoid the risk of hematoma following the injection.

Information for the Patient, Parent, or Guardian

The health care provider should inform the patient, parent, or guardian that vaccination does not substitute for routine cervical cancer screening. Women who receive GARDASIL should continue to undergo cervical cancer screening per standard of care.

The health care provider should provide the vaccine information required to be given with each vaccination to the patient, parent, or guardian.

The health care provider should inform the patient, parent, or guardian of the benefits and risks associated with vaccination. For risks associated with vaccination, see PRECAUTIONS and ADVERSE REACTIONS.

GARDASIL is not recommended for use in pregnant women.

The health care provider should inform the patient, parent, or guardian of the importance of completing the immunization series unless contraindicated.

Patients, parents, or guardians should be instructed to report any adverse reactions to their health care provider.

Drug Interactions

Use with Other Vaccines

Results from clinical studies indicate that GARDASIL may be administered concomitantly (at a separate injection site) with hepatitis B vaccine (recombinant) (see CLINICAL PHARMACOLOGY, *Studies with Other Vaccines*). Co-administration of GARDASIL with other vaccines has not been studied.

Use with Hormonal Contraceptives

In clinical studies, 13,293 subjects (vaccine = 6644; placebo = 6649) who had post-Month 7 follow-up used hormonal contraceptives for a total of 17,597 person-years (65.1% of the total follow-up time in the study for these subjects). Use of hormonal contraceptives or lack of use of hormonal contraceptives among study participants did not alter vaccine efficacy in the PPE population.

Use with Systemic Immunosuppressive Medications

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune responses to vaccines (see PRECAUTIONS, *General*).

Carcinogenesis, Mutagenesis, Impairment of Fertility

GARDASIL has not been evaluated for the potential to cause carcinogenicity or genotoxicity.

GARDASIL administered to female rats at a dose of 120 mcg total protein, which corresponds to approximately 300-fold excess relative to the projected human dose, had no effects on mating performance, fertility, or embryonic/fetal survival.

Pregnancy
Pregnancy Category B:

Reproduction studies have been performed in female rats at doses up to 300 times the human dose (on a mg/kg basis) and have revealed no evidence of impaired female fertility or harm to the fetus due to GARDASIL. However, it is not known whether GARDASIL can cause fetal harm when administered to a pregnant woman or if it can affect reproductive capacity. GARDASIL should be given to a pregnant woman only if clearly needed. An evaluation of the effect of GARDASIL on embryo-fetal, pre- and postweaning development was conducted using rats. One group of rats was administered GARDASIL twice prior to gestation, during the period of organogenesis (gestation day 6) and on lactation day 7. A second group of pregnant rats was administered GARDASIL during the period of organogenesis (gestation day 6) and on lactation day 7 only. GARDASIL was administered at 0.5 mL/rat/occasion (approximately 300-fold excess relative to the projected human dose on a mg/kg basis) by intramuscular injection. No adverse effects on mating, fertility, pregnancy, parturition, lactation, embryo-fetal or pre- and postweaning development were observed. There were no vaccine-related fetal malformations or other evidence of teratogenesis noted in this study. In addition, there were no treatment-related effects on developmental signs, behavior, reproductive performance, or fertility of the offspring. The effect of GARDASIL on male fertility has not been studied.

In clinical studies, women underwent urine pregnancy testing prior to administration of each dose of GARDASIL. Women who were found to be pregnant before completion of a 3-dose regimen of GARDASIL were instructed to defer completion of their vaccination regimen until resolution of the pregnancy.

During clinical trials, 2266 women (vaccine = 1115 vs. placebo = 1151) reported at least 1 pregnancy each. Overall, the proportions of pregnancies with an adverse outcome were comparable in subjects who received GARDASIL and subjects who received placebo. Overall, 40 and 41 subjects in the group that received GARDASIL or placebo, respectively (3.6% and 3.6% of all subjects who reported a pregnancy in the respective vaccination groups), experienced a serious adverse experience during pregnancy. The most common events reported were conditions that can result in Caesarean section (e.g., failure of labor, malpresentation, cephalopelvic disproportion), premature onset of labor (e.g., threatened abortions, premature rupture of membranes), and pregnancy-related medical problems (e.g., pre-eclampsia, hyperemesis). The proportions of pregnant subjects who experienced such events were comparable between the vaccination groups.

There were 15 cases of congenital anomaly in pregnancies that occurred in subjects who received GARDASIL and 16 cases of congenital anomaly in pregnancies that occurred in subjects who received placebo.

Further sub-analyses were conducted to evaluate pregnancies with estimated onset within 30 days or more than 30 days from administration of a dose of GARDASIL or placebo. For pregnancies with estimated onset within 30 days of vaccination, 5 cases of congenital anomaly were observed in the group that received GARDASIL compared to 0 cases of congenital anomaly in the group that received placebo. The congenital anomalies seen in pregnancies with estimated onset within 30 days of vaccination included pyloric stenosis, congenital megacolon, congenital hydronephrosis, hip dysplasia and club foot. Conversely, in pregnancies with onset more than 30 days following vaccination, 10 cases of congenital anomaly were observed in the group that received GARDASIL compared with 16 cases of congenital anomaly in the group that received placebo. The types of anomalies observed were consistent (regardless of when pregnancy occurred in relation to vaccination) with those generally observed in pregnancies in women aged 16 to 26 years.

Pregnancy Registry for GARDASIL

Merck & Co., Inc. maintains a Pregnancy Registry to monitor fetal outcomes of pregnant women exposed to GARDASIL. Patients and health care providers are encouraged to report any exposure to GARDASIL during pregnancy by calling (800) 986-8999.

Lactation

It is not known whether vaccine antigens or antibodies induced by the vaccine are excreted in human milk.

Because many drugs are excreted in human milk, caution should be exercised when GARDASIL is administered to a nursing woman.

A total of 995 nursing mothers (vaccine = 500, placebo = 495) were given GARDASIL or placebo during the vaccination period of the clinical trials. GMTs in nursing and non-nursing mothers were as follows:

The GMTs in nursing mothers were 595.9 (95% CI: 522.5, 679.5) for anti-HPV 6, 864.3 (95% CI: 754.0, 990.8) for anti-HPV 11, 3056.9 (95% CI: 2594.4, 3601.8) for anti-HPV 16, and 527.2 (95% CI: 450.9, 616.5) for anti-HPV 18. The GMTs for women who did not nurse during vaccine administration were 540.1 (95% CI: 523.5, 557.2) for anti-HPV 6, 746.3 (95% CI: 720.4, 773.3) for anti-HPV 11, 2290.8 (95% CI: 2180.7, 2406.3) for anti-HPV 16, and 456.0 (95% CI: 438.4, 474.3) for anti-HPV 18.

Overall, 17 and 9 infants of subjects who received GARDASIL or placebo, respectively (representing 3.4% and 1.8% of the total number of subjects who were breast-feeding during the period in which they received GARDASIL or placebo, respectively), experienced a serious adverse experience. None was judged by the investigator to be vaccine related.

In clinical studies, a higher number of breast-feeding infants (n = 6) whose mothers received GARDASIL had acute respiratory illnesses within 30 days post-vaccination of the mother as compared to infants (n = 2) whose mothers received placebo. In these studies, the rates of other adverse experiences in the mother and the nursing infant were comparable between vaccination groups.

Pediatric Use

The safety and efficacy of GARDASIL have not been evaluated in children younger than 9 years.

Geriatric Use

The safety and efficacy of GARDASIL have not been evaluated in adults above the age of 26 years.

ADVERSE REACTIONS

In 5 clinical trials (4 placebo-controlled), subjects were administered GARDASIL or placebo on the day of enrollment, and approximately 2 and 6 months thereafter. Few subjects (0.1%) discontinued due to adverse experiences. In all except 1 of the clinical trials, safety was evaluated using vaccination report card (VRC)-aided surveillance for 14 days after each injection of GARDASIL or placebo. The subjects who were monitored using VRC-aided surveillance included 5088 girls and women 9 through 26 years of age at enrollment who received GARDASIL and 3790 girls and women who received placebo.

Common Adverse Experiences

Vaccine-related Common Adverse Experiences

The vaccine-related adverse experiences that were observed among female recipients of GARDASIL at a frequency of at least 1.0% and also at a greater frequency than that observed among placebo recipients are shown in Table 6.

Table 6
Vaccine-related Injection-site and Systemic Adverse Experiences*

Adverse Experience (1 to 5 Days Postvaccination)	GARDASIL (N = 5088) %	Aluminum-Containing Placebo (N = 3470) %	Saline Placebo (N = 320) %
Injection Site			
Pain	83.9	75.4	48.6
Swelling	25.4	15.8	7.3
Erythema	24.6	18.4	12.1
Pruritus	3.1	2.8	0.6

Adverse Experience (1 to 15 Days Postvaccination)	GARDASIL (N = 5088) %	Placebo (N = 3790) %
Systemic		
Fever	10.3	8.6

*The vaccine-related adverse experiences that were observed among recipients of GARDASIL were at a frequency of at least 1.0% and also at a greater frequency than that observed among placebo recipients.

All-cause Common Systemic Adverse Experiences

All-cause systemic adverse experiences for female subjects that were observed at a frequency of greater than or equal to 1% where the incidence in the vaccine group was greater than or equal to the incidence in the placebo group are shown in Table 7.

Table 7
All-cause Common Systemic Adverse Experiences

Adverse Experience (1 to 15 Days Postvaccination)	**GARDASIL** (N = 5088) %	**Placebo** (N = 3790) %
Pyrexia	13.0	11.2
Nausea	6.7	6.6
Nasopharyngitis	6.4	6.4
Dizziness	4.0	3.7
Diarrhea	3.6	3.5
Vomiting	2.4	1.9
Myalgia	2.0	2.0
Cough	2.0	1.5
Toothache	1.5	1.4
Upper respiratory tract infection	1.5	1.5
Malaise	1.4	1.2
Arthralgia	1.2	0.9
Insomnia	1.2	0.9
Nasal congestion	1.1	0.9

Evaluation of Injection-site Adverse Experiences by Dose

An analysis of injection-site adverse experiences in female subjects by dose is shown in Table 8. Overall, 94.3% of subjects who received GARDASIL judged their injection-site adverse experience to be mild or moderate in intensity.

Table 8
Postdose Evaluation of Injection-site Adverse Experiences

	Vaccine (% occurrence)				Aluminum-Containing Placebo (% occurrence)				Saline Placebo (% occurrence)			
Adverse Experience	Post-dose 1	Post-dose 2	Post-dose 3	Post Any Dose	Post-dose 1	Post-dose 2	Post-dose 3	Post Any Dose	Post-dose 1	Post-dose 2	Post-dose 3	Post Any Dose
Pain	63.4	60.7	62.7	83.9	57.0	47.8	49.5	75.4	33.7	20.3	27.3	48.6
Mild/Moderate	62.5	59.7	61.2	81.1	56.6	47.3	48.9	74.1	33.3	20.3	27.0	48.0
Severe	0.9	1.0	1.5	2.8	0.4	0.5	0.6	1.3	0.3	0.0	0.3	0.6
Swelling*	10.2	12.8	15.1	25.4	8.2	7.5	7.6	15.8	4.4	3.0	3.3	7.3
Mild/Moderate	9.6	11.9	14.3	23.3	8.0	7.2	7.3	15.2	4.4	3.0	3.3	7.3
Severe	0.6	0.8	0.8	2.0	0.2	0.3	0.2	0.6	0.0	0.0	0.0	0.0
Erythema*	9.2	12.1	14.7	24.7	9.8	8.4	8.9	18.4	7.3	5.3	5.7	12.1
Mild/Moderate	9.0	11.7	14.3	23.7	9.5	8.3	8.8	18.0	7.3	5.3	5.7	12.1
Severe	0.2	0.3	0.4	0.9	0.3	0.1	0.1	0.4	0.0	0.0	0.0	0.0

*Intensity of swelling and erythema was measured by size (inches): Mild = 0 to ≤1; Moderate = >1 to ≤2; Severe = >2.

Evaluation of Fever by Dose

An analysis of fever in girls and women by dose is shown in Table 9.

Table 9
Postdose Evaluation of Fever

	Vaccine (% occurrence)			Placebo (% occurrence)		
Temperature (°F)	Postdose 1	Postdose 2	Postdose 3	Postdose 1	Postdose 2	Postdose 3
≥100 to <102	3.7	4.1	4.4	3.1	3.8	3.6
≥102	0.3	0.5	0.5	0.3	0.4	0.6

Serious Adverse Experiences

A total of 102 subjects out of 21,464 total subjects (9- to 26-year-old girls and women and 9- to 15-year-old boys) who received both GARDASIL and placebo reported a serious adverse experience on Day 1-15 following any vaccination visit during the clinical trials for GARDASIL. The most frequently reported serious adverse experiences for GARDASIL compared to placebo and regardless of causality were:

headache	(0.03% GARDASIL vs. 0.02% Placebo),
gastroenteritis	(0.03% GARDASIL vs. 0.01% Placebo),
appendicitis	(0.02% GARDASIL vs. 0.01% Placebo),
pelvic inflammatory disease	(0.02% GARDASIL vs. 0.01% Placebo).

One case of bronchospasm and 2 cases of asthma were reported as serious adverse experiences that occurred during Day 1-15 of any vaccination visit.

Deaths

Across the clinical studies, 17 deaths were reported in 21,464 male and female subjects. The events reported were consistent with events expected in healthy adolescent and adult populations. The most common cause of death was motor vehicle accident (4 subjects who received GARDASIL and 3 placebo subjects), followed by overdose/suicide (1 subject who received GARDASIL and 2 subjects who received placebo), and pulmonary embolus/deep vein thrombosis (1 subject who received GARDASIL and 1 placebo subject). In addition, there were 2 cases of sepsis, 1 case of pancreatic cancer, and 1 case of arrhythmia in the group that received GARDASIL, and 1 case of asphyxia in the placebo group.

Systemic Autoimmune Disorders

In the clinical studies, subjects were evaluated for new medical conditions that occurred over the course of up to 4 years of follow up. The number of subjects who received both GARDASIL and placebo and developed a new medical condition potentially indicative of a systemic immune disorder is shown in Table 10.

Table 10
Summary of Subjects Who Reported an Incident Condition Potentially Indicative of Systemic Autoimmune Disorder After Enrollment in Clinical Trials of GARDASIL

Potential Autoimmune Disorder	GARDASIL (N = 11,813)	Placebo (N = 9701)
Specific Terms	**3 (0.025%)**	**1 (0.010%)**
Juvenile arthritis	1	0
Rheumatoid arthritis	2	0
Systemic lupus erythematosus	0	1
Other Terms	**6 (0.051%)**	**2 (0.021%)**
Arthritis	5	2
Reactive Arthritis	1	0
N = Number of subjects enrolled		

Safety in Concomitant Use with Other Vaccines

The safety of GARDASIL when administered concomitantly with hepatitis B vaccine (recombinant) was evaluated in a placebo-controlled study. There were no statistically significant higher rates in systemic or injection-site adverse experiences among subjects who received concomitant vaccination compared with those who received GARDASIL or hepatitis B vaccine alone.

Reporting of Adverse Events

The US Department of Health and Human Services has established a Vaccine Adverse Event Reporting System (VAERS) to accept all reports of suspected adverse events after the administration of any vaccine, including but not limited to the reporting of events required by the National Childhood Vaccine Injury Act of 1986. For information or a copy of the vaccine reporting form, call the VAERS toll-free number at 1-800-822-7967 or report on line to www.vaers.hhs.gov.

DOSAGE AND ADMINISTRATION

Dosage

GARDASIL should be administered intramuscularly as 3 separate 0.5-mL doses according to the following schedule:

First dose: at elected date

Second dose: 2 months after the first dose

Third dose: 6 months after the first dose

Method of Administration

GARDASIL should be administered intramuscularly in the deltoid region of the upper arm or in the higher anterolateral area of the thigh.

GARDASIL must not be injected intravascularly. Subcutaneous and intradermal administration have not been studied, and therefore are not recommended.

The prefilled syringe is for single use only and should not be used for more than 1 individual. For single-use vials a separate sterile syringe and needle must be used for each individual.

The vaccine should be used as supplied; no dilution or reconstitution is necessary. The full recommended dose of the vaccine should be used.

Shake well before use. Thorough agitation immediately before administration is necessary to maintain suspension of the vaccine.

After thorough agitation, GARDASIL is a white, cloudy liquid. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Do not use the product if particulates are present or if it appears discolored.

Single-dose Vial Use

Withdraw the 0.5-mL dose of vaccine from the single-dose vial using a sterile needle and syringe free of preservatives, antiseptics, and detergents. Once the single-dose vial has been penetrated, the withdrawn vaccine should be used promptly, and the vial must be discarded.

Prefilled Syringe Use

Inject the entire contents of the syringe.

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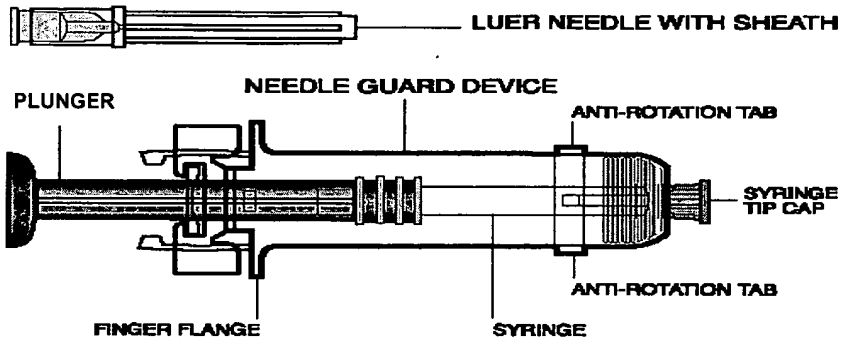
Instructions for using the prefilled single-dose syringes preassembled with needle guard (safety) device



NOTE: Please use the enclosed needle for administration. If a different needle is chosen, it should fit securely on the syringe and be no longer than 1 inch to ensure proper functioning of the needle guard device. Two detachable labels are provided which can be removed after the needle is guarded.

Remove Syringe Tip Cap. Attach Luer Needle. Depress both Anti-Rotation Tabs to secure syringe and attach Luer Needle by twisting in clockwise direction. **Remove Needle Sheath. Administer injection** per standard protocol as stated above under DOSAGE AND ADMINISTRATION. Depress the Plunger while grasping the Finger Flange **until the entire dose has been given.** The Needle Guard Device will **NOT** activate to cover and protect the needle unless the **ENTIRE** dose has been given. Remove needle from the vaccine recipient. Release the Plunger and allow syringe to move up until the entire needle is guarded. For documentation of vaccination, remove detachable labels by pulling slowly on them. **Dispose in approved sharps container.**

HOW SUPPLIED

Vials

No. 4045 — GARDASIL is supplied as a carton of one 0.5-mL single-dose vial, **NDC** 0006-4045-00.

No. 4045 — GARDASIL is supplied as a carton of ten 0.5-mL single-dose vials, **NDC** 0006-4045-41.

Syringes

No. 4109 — GARDASIL is supplied as a carton of one 0.5-mL single-dose prefilled Luer Lock syringe, preassembled with UltraSafe Passive®† delivery system. A one-inch, 25-gauge needle is provided separately in the package. **NDC** 0006-4109-31.

No. 4109 — GARDASIL is supplied as a carton of six 0.5-mL single-dose prefilled Luer Lock syringes, preassembled with UltraSafe Passive® delivery system. One-inch, 25-gauge needles are provided separately in the package. **NDC** 0006-4109-06.

Storage

Store refrigerated at 2 to 8°C (36 to 46°F). Do not freeze. Protect from light.

Manuf. and Dist. by:
MERCK & CO., INC., Whitehouse Station, NJ 08889, USA

Issued June 2006

† UltraSafe Passive® delivery system is a Trademark of Safety Syringes, Inc.

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Printed in USA

USPPI
Patient Information about
GARDASIL® (pronounced "gard-Ah-sill")
Generic name: [Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine]

Read this information with care before you or your child gets GARDASIL*. You or your child will need 3 doses of the vaccine. It is important to read this leaflet when you receive each dose. This leaflet does not take the place of talking with your health care professional about GARDASIL.

What is GARDASIL and what is it used for?
GARDASIL is a vaccine (injection/shot) that helps protect against the following diseases caused by Human Papillomavirus (HPV) Types in the vaccine (6, 11, 16, and 18):

- Cervical cancer (cancer of the lower end of the uterus or womb).
- Abnormal and precancerous cervical lesions.
- Abnormal and precancerous vaginal lesions.
- Abnormal and precancerous vulvar lesions.
- Genital warts.

GARDASIL helps prevent these diseases – but it will not treat them.

You or your child cannot get these diseases from GARDASIL.

What other key information about GARDASIL should I know?

- Vaccination does not substitute for routine cervical cancer screening. Females who receive GARDASIL should continue cervical cancer screening.
- As with all vaccines, GARDASIL may not fully protect everyone who gets the vaccine.
- Gardasil will not protect against diseases due to non-vaccine HPV types. There are more than 100 HPV types; GARDASIL helps protect against 4 types (6, 11, 16, and 18). These 4 types have been selected for GARDASIL because they cause approximately 70% of cervical cancers and 90% of genital warts.
- This vaccine will not protect you against HPV types to which you may have already been exposed.
- GARDASIL also will not protect against other diseases that are not caused by HPV.
- GARDASIL works best when given before you or your child has any contact with certain types of HPV (i.e., HPV types 6, 11, 16, and 18).

Who can receive GARDASIL?
GARDASIL is for girls and women 9 through 26 years of age.

See "Who should not receive GARDASIL?" below.

Who should not receive GARDASIL?
Anyone who:

- is allergic to any of the ingredients in the vaccine. A list of ingredients can be found at the end of this leaflet.
- has an allergic reaction after getting a dose of the vaccine.

What should I tell my health care professional before I am vaccinated or my child is vaccinated with GARDASIL?
It is very important to tell your health care professional if you or your child:

- has had an allergic reaction to the vaccine.
- has a bleeding disorder and cannot receive injections in the arm.
- has a weakened immune system, for example, due to a genetic defect or HIV infection.
- is pregnant or is planning to get pregnant. GARDASIL is not recommended for use in pregnant women.
- has any illness with a fever more than 100°F (37.8°C).
- takes or plans to take any medicines, even those you can buy over the counter.

Your health care professional will decide if you or your child should receive the vaccine.

How is GARDASIL given?
GARDASIL is given as an injection.

You or your child will receive 3 doses of the vaccine. Ideally the doses are given as:
- First dose: at a date you and your health care professional choose.
- Second dose: 2 months after the first dose.
- Third dose: 6 months after the first dose.

Make sure that you or your child gets all 3 doses. This allows you or your child to get the full benefits of GARDASIL. If you or your child misses a dose, your health care professional will decide when to give the missed dose.

What are the possible side effects of GARDASIL?
As with all vaccines, there may be some side effects with GARDASIL. GARDASIL has been shown to be generally well tolerated in women and girls as young as 9 years of age.

The most commonly reported side effects included:
- pain, swelling, itching, and redness at the injection site.
- fever.

Difficulty breathing (bronchospasm) has been reported very rarely.

If you or your child has any unusual or severe symptoms after receiving GARDASIL, contact your health care professional right away.

For a more complete list of side effects, ask your health care professional.

What are the ingredients in GARDASIL?
The main ingredients are purified inactive proteins that come from HPV Types 6, 11, 16, and 18.

It also contains amorphous aluminum hydroxyphosphate sulfate, sodium chloride, L-histidine, polysorbate 80, sodium borate, and water for injection.

What are cervical cancer, precancerous lesions, and genital warts?
Cancer of the cervix is a serious disease that can be life-threatening. This disease is caused by certain HPV types that can cause the cells in the lining of the cervix to change from normal to precancerous lesions. If these are not treated, they can turn cancerous.

Genital warts are caused by certain types of HPV. They often appear as skin-colored growths. They are found on the inside or outside of the genitals. They can hurt, itch, bleed, and cause discomfort. These lesions are usually not precancerous. Sometimes, it takes multiple treatments to eliminate these lesions.

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What is Human Papillomavirus (HPV)?
HPV is a common virus. In 2005, the Centers for Disease Control and Prevention (CDC) estimated that 20 million people in the United States had this virus. There are many different types of HPV; some cause no harm. Others can cause diseases of the genital area. For most people the virus goes away on its own. When the virus does not go away it can develop into cervical cancer, precancerous lesions, or genital warts, depending on the HPV type. See "What other key information about GARDASIL should I know?"

Who is at risk for Human Papillomavirus?
In 2005, the CDC estimated that at least 50% of sexually active people catch HPV during their lifetime. A male or female of any age who takes part in any kind of sexual activity that involves genital contact is at risk.

Many people who have HPV may not show any signs or symptoms. This means that they can pass on the virus to others and not know it.

Will GARDASIL help me if I already have Human Papillomavirus?
You may benefit from GARDASIL if you already have HPV. This is because most people are not infected with all four types of HPV contained in the vaccine. In clinical trials, individuals with current or past infection with one or more vaccine-related HPV types prior to vaccination were protected from disease caused by the remaining vaccine HPV types. GARDASIL is not intended to be used for treatment for the above mentioned diseases. Talk to your health care professional for more information.

This leaflet is a summary of information about GARDASIL. If you would like more information, please talk to your health care professional or visit www.gardasil.com.

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Whitehouse Station, NJ 08889, USA

EXHIBIT 3
8 PAGES

EXHIBIT 3

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852-1448

JUN - 8 2006

Our STN: BL 125126/0

Merck & Co., Inc.
Attn: Dr. Patrick Brill-Edwards
Director
Worldwide Regulatory Affairs
Vaccines/Biologics
P.O. Box 4, BLB-22
West Point, PA 19486-0004

Dear Dr. Brill-Edwards:

We have approved your biologics license application (BLA) for Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine under your existing Department of Health and Human Services U.S. License No. 0002. Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine is indicated for vaccination in females 9 to 26 years of age for prevention of the following diseases caused by Human Papillomavirus (HPV) Types 6, 11, 16, and 18:

- Cervical cancer
- Genital warts (condyloma acuminata)

and the following precancerous or dysplastic lesions:

- Cervical adenocarcinoma *in situ* (AIS)
- Cervical intraepithelial neoplasia (CIN) grade 2 and grade 3
- Vulvar intraepithelial neoplasia (VIN) grade 2 and grade 3
- Vaginal intraepithelial neoplasia (VaIN) grade 2 and grade 3
- Cervical intraepithelial neoplasia (CIN) grade 1.

Under this authorization, you are approved to manufacture Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine at Merck & Co., Inc., West Point, PA. The final formulation and filling is performed by Merck & Co., Inc., West Point, PA. Labeling and packaging will be performed by Merck & Co., Inc., West Point, PA. You may label your product with the proprietary name GARDASIL®. The vaccine will be supplied as a 0.5 mL single-dose vial, a carton of ten 0.5 mL single dose vials, a 0.5 mL single-dose prefilled syringe and a carton of six 0.5 mL single-dose prefilled syringes. The prefilled syringes will be preassembled with Ultra Safe® Passive™ Needle Guard devices.

The dating period for GARDASIL® vaccine shall be 36 months from the date of manufacture of the final filled container when stored at 2 to 8 °C. The 36 month shelf life includes all of the time that the final filled container is held at 2 to 8 °C prior to packaging. The date of manufacture shall be defined as the start date of filling into final containers. The monovalent bulk lots used to formulate the final container vaccine shall be held for no longer than 36 months at 2 to 8 °C.

Please submit final bulk samples and final container samples of the product together with lot release protocols in electronic format showing results of all applicable tests. You may not distribute any lots of product until you receive a notification of release from the Director, Center for Biologics Evaluation and Research (CBER).

You must submit information to your BLA for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of GARDASIL® vaccine, or in the manufacturing facilities.

Under the Pediatric Research Equity Act (PREA), all applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are deferring pediatric studies for GARDASIL® in girls less than 9 years of age and in boys and adolescent males less than 18 years of age.

Postmarketing Studies subject to reporting requirements of 21 CFR 601.70.

We acknowledge the postmarketing clinical commitments outlined in your submission of June 6, 2006, as follows:

1. You have committed to conduct a short-term safety surveillance study in a U.S. Managed Care Organization (MCO). The study will include approximately 44,000 vaccinated subjects who will be followed for 60 days for assessment of general short-term safety (i.e., emergency room visits, hospitalizations, and deaths). The subjects will also be followed for 6 months subsequent to vaccination for new autoimmune disorders, rheumatologic conditions, or thyroiditis. Also, a sufficient number of children 11-12 years of age will be studied to permit an analysis of safety outcomes. The final study protocol will be submitted by December 31, 2006. Patient accrual will be completed by December 31, 2008. The study will be completed by June 30, 2009. The final study report will be submitted by September 30, 2009.

2. You have committed to collaborate with the cancer registries in four countries in the Nordic Region (Sweden, Norway, Iceland, and Denmark) to assess long-term outcomes following administration of GARDASIL®. In this study, approximately 5,500 subjects enrolled in Protocol 015 (one half from the placebo group that will have been vaccinated shortly after approval) will be followed for a total of 14 years. Two major goals of this study are: 1) to assess the long-term effectiveness of GARDASIL® by evaluating biopsy specimens for presence of HPV 6/11/16/18-related incident breakthrough cases of

CIN 2/3, AIS and cervical cancer, VIN 2/3 and vulvar cancer, and VaIN 2/3 and vaginal cancer; and 2) to assess whether administration of GARDASIL® will result in replacement of these diseases due to vaccine HPV types with diseases due to non-vaccine HPV types. This study is designed to accomplish these goals as discussed in the June 6, 2006, submission to your BLA. The final protocol for this study will be submitted by December 8, 2006. Patient accrual for this study was previously completed in the context of Protocol 015. This study will be completed by December 31, 2017, (14 years from initiation of the last patient enrolled in Protocol 015 in the four Nordic countries). The final study report will be submitted by December 31, 2018.

3. You have committed to conduct a study in collaboration with the Norwegian Government, if GARDASIL® is approved in the European Union and the Government of Norway incorporates HPV vaccination into its national guidelines, to assess the impact of HPV vaccination on the following in Norway:

   a. The long-term burden of HPV disease including the incidence of HPV 6/11/16/18-related cervical disease;
   b. The long-term burden of HPV disease caused by types other than HPV 6/11/16/18;
   c. The overall incidence of cervical HPV disease;
   d. The incidence of HPV-related cancers and pre-cancers (CIN 2/3, AIS and cervical cancer; VIN 2/3 and vulvar cancer; and VaIN 2/3 and vaginal cancer);
   e. The interaction between administration of GARDASIL® and pregnancy outcomes, especially congenital anomalies, by linking the vaccination registry with the Medical Birth Registry.

   The size and age range of the population studied will depend on the final vaccination guidelines implemented by the Norwegian Government. Although at this time no other governments in the Nordic region have committed to similar population studies, you will notify CBER of any other collaborations if they occur. The projected date of submission of the final study protocol is pending collaboration with the Norwegian Government as noted above. Patient accrual will be completed 6 years after study initiation. The study will be completed 7 years after study initiation. The final study report will be submitted 8 years after study initiation. In the event that approval of GARDASIL® does not occur in Norway, you will notify CBER and propose alternative approaches to obtain this information in a timely manner.

4. You have committed to submit final Clinical Study Reports (CSRs) for Protocols 013 and 015 when completed. As discussed, for these studies, an "all CIN 2/3, AIS or cervical cancer" analysis will evaluate the evidence for replacement of disease due to HPV types 16 and 18 with non-vaccine HPV types. Similar analyses will be done for VIN 2/3, VaIN 2/3, vulvar cancer and vaginal cancer. Protocol 013 was submitted in December 2001, and Protocol 015 was submitted in May 2002. Protocol 013 accrual was completed in March 2003, and Protocol 015 accrual was completed in May 2003. These analyses will be completed by April 30, 2007. The final reports for these studies (i.e., CSRs) to include the results of these analyses will be submitted by June 30, 2007.

5. You have committed to provide data concerning duration of immunity following administration of GARDASIL® as follows from the studies noted:

   a. The Nordic Long-Term Follow-up Study:
   Interim reports of effectiveness (i.e., incident breakthrough cases of CIN 2/3, AIS and cervical cancer; VIN 2/3 and vulvar cancer; and VaIN 2/3 and vaginal cancer) and immunogenicity results will be submitted in 2009, 2011, 2013, and 2015. The final study report will be submitted by December 31, 2018.

   b. Protocol 018 (Adolescent Sentinel Cohort):
   - Periodic reports beginning with Month 24 immunogenicity and long-term safety data will be submitted starting no later than March 30, 2007.
   - Publication of one year Post-dose 3 data will be submitted by January 30, 2007.
   - A Biologics License Supplement (BLS) for 1.5 year Post-dose 3 data will be submitted by June 30, 2007.
   - A Biologics License Supplement (BLS) for 2.5 year Post-dose 3 data will be submitted by December 31, 2007.
   - A Biologics License Supplement (BLS) for 5.5 year Post-dose 3 data will be submitted by December 31, 2010.

   c. Protocol 007:
   Publication of five-year immunogenicity data will be submitted by December 31, 2006.

   d. Protocol 005:
   Publication of seven and one half year immunogenicity data will be submitted by December 31, 2007.

6. You have agreed to establish a pregnancy registry in the U.S. to prospectively collect data on spontaneously-reported exposures to GARDASIL® during pregnancy. You have committed to submit a protocol for the U.S. pregnancy registry by July 20, 2006. You have agreed to address elements found in FDA's Guidance for Industry on Establishing Pregnancy Exposure Registries (9/2/2002) (http://www.fda.gov/cber/gdlns/pregexp.htm), as well as relevant Company Standard Operating Procedures. Furthermore, you have stated that you will notify CBER of significant deviations from this guidance and/or specify the deviations in the protocol. Patient accrual/data collection will begin at time of CBER's approval of the protocol and end five years later. You will submit annual reports and a final summary report of the U.S. pregnancy registry's findings five years after initiation of patient accrual/data collection. The U.S. pregnancy database will be considered completed one month after discontinuation of patient accrual for the purpose of preparing a five-year final summary report. The five-year final summary report will be submitted to CBER five years and six months after initiation of patient accrual/data collection. After reviewing the five-year data, Merck and CBER will meet to discuss the

need to continue further data collection in the U.S. pregnancy registry. CBER will have final approval regarding any decision to discontinue the U.S. pregnancy registry.

Postmarketing Studies not subject to reporting requirements of 21 CFR 601.70.

We acknowledge the postmarketing clinical commitment outlined in your submission of June 6, 2006, as follows:

7. You have committed to provide CBER and simultaneously the FDA contractor for the Vaccine Adverse Events Reporting System (VAERS) all initial postmarketing "periodic" adverse experience reports received that are subject to periodic reporting (i.e., not covered under the "15-day Alert report" requirement under 21 CFR 600.80) on a monthly basis. Initial reports received by Merck in a given month will be submitted on VAERS forms to CBER and to the VAERS contractor by Working Day 10 of the following month. You have also agreed to provide, in accordance with 21 CFR 600.80, the Quarterly Periodic Adverse Experience Report to the VAERS contractor. The Quarterly Adverse Experience Report will contain a recapitulation of all initial reports submitted for the current reporting period and will include all follow up information on VAERS forms collected during that three-month period. You have committed to providing CBER this information using the aforementioned process, for the first three years after the date of licensure.

We acknowledge the postmarketing quality commitments outlined in your submission of June 2, 2006, as follows:

8. You have committed to submitting a proposal for establishing upper limits for the *in vitro* relative potency assay (IVRP) for HPV 6, 11, 16, and 18 for the Quadrivalent Final Container Product (QFCP). The upper quality control limits will be based on data obtained from full-scale manufacturing experience for the IVRP assay for each HPV type in the QFCP. The proposal will be submitted by July 31, 2006, and will also describe how lots with IVRP assay results that exceed these limits will be handled.

9. You have committed to providing stability data through the 48-month time point for the ongoing cell slurry stability study for HPV 6, 11, 16, and 18 by July 31, 2006.

10. You have committed to providing all currently available stability data for Monovalent Bulk Adsorbed Product (MBAP) Lots for HPV 6, 11, 16, and 18 to CBER by July 31, 2006. These MBAP lots are currently on full or abridged stability studies at 2 to 8 °C.

11. You have committed to providing the final study data for the HPV 6, 11, 16, and 18 MBAP lots on the accelerated stability study at 23 to 27 °C by July 31, 2006.

12. You have committed to providing all available stability data on all final container lots (both vials and syringes) currently on stability study to support the requested product shelf-life of 36 months at 2 to 8 °C by July 31, 2006. Additional updates to the stability

data for these lots will be submitted as they become available, through the study endpoint (42 months storage at 2 to 8 °C).

13. You have committed to providing all available stability data on all final container lots (both vials and syringes) used in the accelerated stability study at 23 to 27 °C by July 31, 2006. Additional updates to the stability data for these lots will be submitted as they become available, through the study endpoint (12 months storage at 23 to 27 °C).

14. You have committed to providing stability data from the sequential stability study. The stability data on the MBAP lots are covered by the commitment made in item 10 above as these lots were also used for the real time MBAP stability studies. Stability data on the QFCP lot formulated from MBAP lots stored at 2 to 8 °C for a minimum of 36 months will be submitted on an annual basis, at the time of the Annual Report submissions, through the completion of the study.

15. You have committed to providing study data for the first three full-scale lots filled into glass vials with Teflon-2 stoppers. Data from these final container stability studies will be submitted as they become available, through the study endpoint. However, we acknowledge that you currently do not have any plans to fill full-scale lots in glass vials with Teflon-2 stoppers.

16. You have committed to continue testing for Completeness of Adsorption (COA) on the QFCP until 50 full scale final container lots, representing 50 formulation lots, have been tested. You will submit COA data for these 50 lots to CBER as a CBE following licensure. CBER will evaluate the data and determine whether COA testing for lot release can be discontinued.

We request that you submit clinical protocols to your IND 9030, with a cross-reference letter to this biologics license application (BLA), STN BL 125126. We request that you submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to your BLA, STN BL 125126. Please use the following designators to prominently label all submissions, including supplements, relating to these postmarketing study commitments, as appropriate:

- **Postmarketing Study Protocol**
- **Postmarketing Study Final Report**
- **Postmarketing Study Correspondence**
- **Annual Report on Postmarketing Studies**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e., pending, ongoing, delayed, terminated, or submitted), and

- an explanation of the status including, for clinical studies, the subject accrual rate (i.e., number enrolled to date and the total planned enrollment).

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to FDA's Guidance for Industry: Reports on the Status of Postmarketing Study Commitments – Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (February 2006) (see http://www.fda.gov/cber/gdlns/post130.htm) for further information.

Please submit adverse experience reports in accordance with the adverse experience reporting requirements for licensed biological products (21 CFR 600.80), and distribution reports as described in (21 CFR 600.81). Under 21 CFR 600.80(c) (2) [Periodic Adverse Experience Reports], you must report each adverse experience not reported under paragraph (c) (1) (i) of this section at quarterly intervals for the first 3 years following approval, and then at annual intervals. We note your clinical commitment in item 7 above to submit certain reports on a monthly basis for the first three years following approval. Since your product is characterized as a vaccine, submit these reports to the Vaccine Adverse Event Reporting System (VAERS) using the pre-addressed form VAERS-1.

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Director, Office of Compliance and Biologics Quality, Center for Biologics Evaluation and Research, HFM-600, 1401 Rockville Pike, Rockville, MD 20852-1448.

Please submit all final printed labeling and implementation information on FDA Form 356h. Please provide a PDF-format electronic version of the label.

In addition, you may wish to submit two draft copies of the proposed introductory advertising and promotional labeling with an FDA Form 2253 to the Center for Biologics Evaluation and Research, Advertising and Promotional Labeling Branch, HFM-602, 1401 Rockville Pike, Rockville, MD 20852-1448. Two copies of final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253. All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have submitted data to support such claims to us and received CBER approval for such claims.

If you have any questions, please contact Dr. Gopa Raychaudhuri at 301-827-3070.

Sincerely yours,

Norman W. Baylor, Ph.D.
Director
Office of Vaccines
Research and Review
Center for Biologics
Evaluation and Research

EXHIBIT 4
26 PAGES

EXHIBIT 4

US005820870A

United States Patent [19]

Joyce et al.

[11] Patent Number: 5,820,870

[45] Date of Patent: Oct. 13, 1998

[54] **RECOMBINANT HUMAN PAPILLOMAVIRUS TYPE 18 VACCINE**

[75] Inventors: **Joseph G. Joyce**, Lansdale; **Hugh A. George**, Schwenksville; **Kathryn J. Hofman**, Collegeville; **Kathrin U. Jansen**, Ft. Washington; **Michael P. Neeper**, Collegeville, all of Pa.

[73] Assignee: **Merck & Co., Inc.**, Rahway, N.J.

[21] Appl. No.: **409,122**

[22] Filed: **Mar. 22, 1995**

[51] **Int. Cl.**[6] **A61K 39/02**; A61K 39/00; C12P 21/06; C07K 1/00

[52] **U.S. Cl.** **424/204.1**; 424/186.1; 424/184.1; 435/64.3; 435/69.1; 435/235.1; 435/254.2; 530/350; 530/412; 530/23.72

[58] **Field of Search** 435/69.3, 69.1, 435/235.1, 254.2; 424/204.1, 186.1, 184.1; 530/350, 412, 23.72

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Primary Examiner—Marian C. Knode
Assistant Examiner—Ali R. Salimi
Attorney, Agent, or Firm—Joanne M. Giesser; Jack L. Tribble

[57] **ABSTRACT**

The present invention is directed to vaccines for human papillomavirus type 18 and derivatives thereof.

11 Claims, 7 Drawing Sheets

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          10         20         30         40         50         60
ATGGCTTTGTGGCGGCCTAGTGACAATACCGTATACCTTCCACCTCCTTCTGTGGCAAGA
  1 M  A  L  W  R  P  S  D  N  T  V  Y  L  P  P  P  S  V  A  R    20
          70         80         90        100        110        120
GTTGTAAATACTGATGATTATGTGACTCGCACAAGCATATTTTATCATGCTGGCAGCTCT
 21 V  V  N  T  D  D  Y  V  T  R  T  S  I  F  Y  H  A  G  S  S    40
         130        140        150        160        170        180
AGATTATTAACTGTTGGTAATCCATATTTTAGGGTTCCTGCAGGTGGTGGCAATAAGCAG
 41 R  L  L  T  V  G  N  P  Y  F  R  V  P  A  G  G  G  N  K  Q    60
         190        200        210        220        230        240
GATATTCCTAAGGTTTCTGCATACCAATATAGAGTATTTCGGGTGCAGTTACCTGACCCA
 61 D  I  P  K  V  S  A  Y  Q  Y  R  V  F  R  V  Q  L  P  D  P    80
         250        260        270        280        290        300
AATAAATTTGGTTTACCTGATAATAGTATTTATAATCCTGAAACACAACGTTTAGTGTGG
 81 N  K  F  G  L  P  D  N  S  I  Y  N  P  E  T  Q  R  L  V  W   100
         310        320        330        340        350        360
GCCTGTGCTGGAGTGGAAATTGGCCGTGGTCAGCCTTTAGGTGTTGGCCTTAGTGGGCAT
101 A  C  A  G  V  E  I  G  R  G  Q  P  L  G  V  G  L  S  G  H   120
         370        380        390        400        410        420
CCATTTTATAATAAATTAGATGACACTGAAAGTTCCCATGCCGCTACGTCTAATGTTTCT
121 P  F  Y  N  K  L  D  D  T  E  S  S  H  A  A  T  S  N  V  S   140
         430        440        450        460        470        480
GAGGACGTTAGGGACAATGTGTCTGTAGATTATAAGCAGACACAGTTATGTATTTTGGGC
141 E  D  V  R  D  N  V  S  V  D  Y  K  Q  T  Q  L  C  I  L  G   160
         490        500        510        520        530        540
TGTGCCCCTGCTATTGGGGAACACTGGGCTAAAGGCACTGCTTGTAAATCGCGTCCTTTA
161 C  A  P  A  I  G  E  H  W  A  K  G  T  A  C  K  S  R  P  L   180
         550        560        570        580        590        600
TCACAGGGCGATTGCCCCCCTTTAGAACTTAAGAACACAGTTTTGGAAGATGGTGATATG
181 S  Q  G  D  C  P  P  L  E  L  K  N  T  V  L  E  D  G  D  M   200
         610        620        630        640        650        660
GTAGATACTGGATATGGTGCCATGGACTTTAGTACATTGCAAGATACTAAATGTGAGGTA
201 V  D  T  G  Y  G  A  M  D  F  S  T  L  Q  D  T  K  C  E  V   220
         670        680        690        700        710        720
CCATTGGATATTTGTCAGTCTATTTGTAAATATCCTGATTATTTACAAATGTCTGCAGAT
221 P  L  D  I  C  Q  S  I  C  K  Y  P  D  Y  L  Q  M  S  A  D   240
         730        740        750        760        770        780
CCTTATGGGGATTCCATGTTTTTTTGCTTACGACGTGAGCAGCTTTTTGCTAGGCATTTT
241 P  Y  G  D  S  M  F  F  C  L  R  R  E  Q  L  F  A  R  H  F   260
         790        800        810        820        830        840
TGGAATAGGGCAGGTACTATGGGTGACACTGTGCCTCAATCCTTATATATTAAAGGCACA
261 W  N  R  A  G  T  M  G  D  T  V  P  Q  S  L  Y  I  K  G  T   280
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FIG. 1A

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                850       860       870       880       890       900
    GGTATGCGTGCTTCACCTGGCAGCTGTGTGTATTCTCCCTCTCCAAGTGGCTCTATTGTT
281 G M R A S P G S C V Y S P S P S G S I V 300
                910       920       930       940       950       960
    ACCTCTGACTCCCAGTTGTTTAATAAACCATATTGGTTACATAAGGCACAGGGTCATAAC
301 T S D S Q L F N K P Y W L H K A Q G H N 320
                970       980       990      1000      1010      1020
    AATGGTATCTGCTGGCATAATCAATTATTTGTTACTGTGGTAGATACCACTCGTAGTACC
321 N G I C W H N Q L F V T V V D T T R S T 340
               1030      1040      1050      1060      1070      1080
    AATTTAACAATATGTGCTTCTACACAGTCTCCTGTACCTGGGCAATATGATGCTACCAAA
341 N L T I C A S T Q S P V P G Q Y D A T K 360
               1090      1100      1110      1120      1130      1140
    TTTAAGCAGTATAGCAGACATGTTGAAGAATATGATTTGCAGTTTATTTTTCAGTTATGT
361 F K Q Y S R H V E E Y D L Q F I F Q L C 380
               1150      1160      1170      1180      1190      1200
    ACTATTACTTTAACTGCAGATGTTATGTCCTATATTCATAGTATGAATAGCAGTATTTTA
381 T I T L T A D V M S Y I H S M N S S I L 400
               1210      1220      1230      1240      1250      1260
    GAGGATTGGAACTTTGGTGTTCCCCCCCCGCCAACTACTAGTTTGGTGGATACATATCGT
401 E D W N F G V P P P P T T S L V D T Y R 420
               1270      1280      1290      1300      1310      1320
    TTTGTACAATCTGTTGCTATTACCTGTCAAAAGGATGCTGCACCAGCTGAAAATAAGGAT
421 F V Q S V A I T C Q K D A A P A E N K D 440
               1330      1340      1350      1360      1370      1380
    CCCTATGATAAGTTAAAGTTTTGGAATGTGGATTTAAAGGAAAAGTTTTCTTTGGACTTA
441 P Y D K L K F W N V D L K E K F S L D L 460
               1390      1400      1410      1420      1430      1440
    GATCAATATCCCCTTGGACGTAAATTTTTGGTTCAGGCTGGATTGCGTCGCAAGCCCACC
461 D Q Y P L G R K F L V Q A G L R R K P T 480
               1450      1460      1470      1480      1490      1500
    ATAGGCCCTCGTAAACGTTCTGCTCCATCTGCCACTACGTCTTCTAAACCTGCCAAGCGT
481 I G P R K R S A P S A T T S S K P A K R 500
               1510      1520
    GTGCGTGTACGTGCCAGGAAGTAA
501 V R V R A R K * 508
```

FIG. 1B

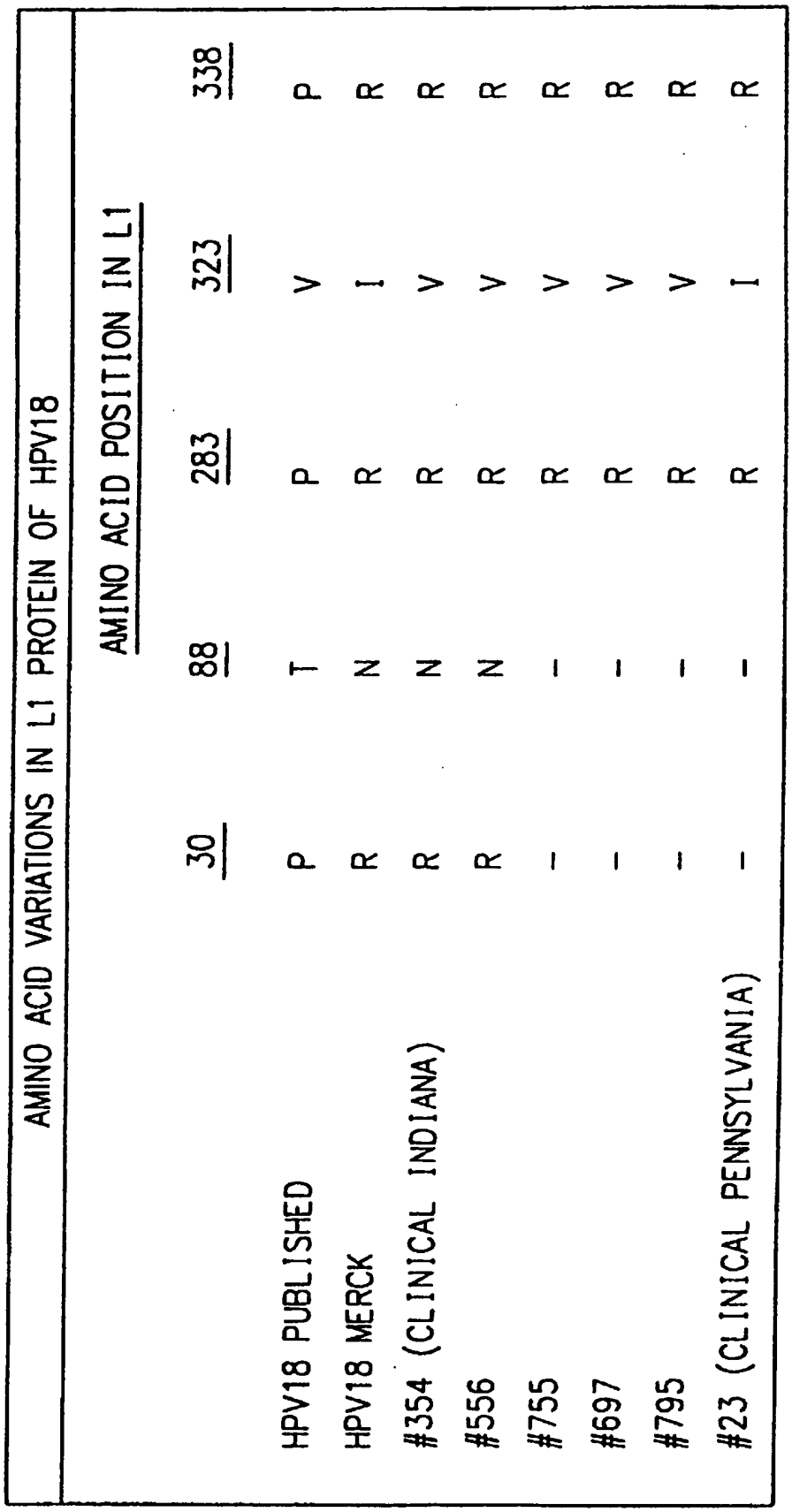
AMINO ACID VARIATIONS IN L1 PROTEIN OF HPV18
AMINO ACID POSITION IN L1
30 88 283 323 338
HPV18 PUBLISHED P T P V P
HPV18 MERCK R N R I R
#354 (CLINICAL INDIANA) R N R V R
#556 R N R V R
#755 – – R V R
#697 – – R V R
#795 – – R V R
#23 (CLINICAL PENNSYLVANIA) – – R I R

FIG.2

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                10        20        30        40        50        60
    ATGGTATCCCACCGTGCCGCACGACGCAAACGGGCTTCGGTGACTGACTTATATAAAACA
 1  M  V  S  H  R  A  A  R  R  K  R  A  S  V  T  D  L  Y  K  T   20
                70        80        90       100       110       120
    TGTAAACAATCTGGTACATGTCCATCTGATGTTGTTAATAAGGTAGAGGGCACCACGTTA
21  C  K  Q  S  G  T  C  P  S  D  V  V  N  K  V  E  G  T  T  L   40
               130       140       150       160       170       180
    GCAGATAAAATATTGCAATGGTCAAGCCTTGGTATATTTTTGGGTGGACTTGGCATAGGT
41  A  D  K  I  L  Q  W  S  S  L  G  I  F  L  G  G  L  G  I  G   60
               190       200       210       220       230       240
    ACTGGAAGTGGTACAGGGGGTCGTACAGGGTACATTCCATTGGGTGGGCGTTCCAATACA
61  T  G  S  G  T  G  G  R  T  G  Y  I  P  L  G  G  R  S  N  T   80
               250       260       270       280       290       300
    GTTGTGGATGTCGGTCCTACACGTCCTCCAGTGGTTATTGAACCTGTGGGCCCCACAGAC
81  V  V  D  V  G  P  T  R  P  P  V  V  I  E  P  V  G  P  T  D  100
               310       320       330       340       350       360
    CCATCTATTGTTACATTAATAGAGGACTCAAGTGTTGTTACATCAGGTGCACCTAGGCCT
101 P  S  I  V  T  L  I  E  D  S  S  V  V  T  S  G  A  P  R  P  120
               370       380       390       400       410       420
    ACTTTTACTGGCACGTCTGGGTTTGATATAACATCTGCTGGTACAACTACACCTGCAGTT
121 T  F  T  G  T  S  G  F  D  I  T  S  A  G  T  T  T  P  A  V  140
               430       440       450       460       470       480
    TTGGATATCACACCTTCGTCTACCTCTGTTTCTATTTCCACAACCAATTTTACCAATCCT
141 L  D  I  T  P  S  S  T  S  V  S  I  S  T  T  N  F  T  N  P  160
               490       500       510       520       530       540
    GCATTTTCTGATCCGTCCATTATTGAAGTTCCACAAACTGGGGAGGTGTCAGGTAATGTA
161 A  F  S  D  P  S  I  I  E  V  P  Q  T  G  E  V  S  G  N  V  180
               550       560       570       580       590       600
    TTTGTTGGTACCCCTACATCTGGAACACATGGGTATGAAGAAATACCTTTACAAACATTT
181 F  V  G  T  P  T  S  G  T  H  G  Y  E  E  I  P  L  Q  T  F  200
               610       620       630       640       650       660
    GCTTCTTCTGGTACGGGGGAGGAACCCATTAGTAGTACCCCATTGCCTACTGTGCGGCGT
201 A  S  S  G  T  G  E  E  P  I  S  S  T  P  L  P  T  V  R  R  220
               670       680       690       700       710       720
    GTAGCAGGTCCCCGCCTTTACAGTAGGGCCTACCAACAAGTGTCTGTGGCTAACCCTGAG
221 V  A  G  P  R  L  Y  S  R  A  Y  Q  Q  V  S  V  A  N  P  E  240
               730       740       750       760       770       780
    TTTCTTACACGTCCATCCTCTTTAATTACCTATGACAACCCGGCCTTTGAGCCTGTGGAC
241 F  L  T  R  P  S  S  L  I  T  Y  D  N  P  A  F  E  P  V  D  260
               790       800       810       820       830       840
    ACTACATTAACATTTGAGCCTCGTAGTAATGTTCCTGATTCAGATTTTATGGATATTATC
261 T  T  L  T  F  E  P  R  S  N  V  P  D  S  D  F  M  D  I  I  280
```

FIG. 3A

```
          850       860       870       880       890       900
     CGTTTACATAGGCCTGCTTTAACATCCAGGCGTGGTACTGTGCGCTTTAGTAGATTAGGT
281  R  L  H  R  P  A  L  T  S  R  R  G  T  V  R  F  S  R  L      300
          910       920       930       940       950       960
     CAAAGGGCAACTATGTTTACCCGTAGCGGTACACAAATAGGTGCTAGGGTTCACTTTTAT
301  Q  R  A  T  M  F  T  R  S  G  T  Q  I  G  A  R  V  H  F  Y  320
          970       980       990      1000      1010      1020
     CATGATATAAGTCCTATTGCACCCTCCCCAGAATATATTGAACTGCAGCCTTTAGTATCT
321  H  D  I  S  P  I  A  P  S  P  E  Y  I  E  L  Q  P  L  V  S  340
         1030      1040      1050      1060      1070      1080
     GCCACGGAGGACAATGGCTTGTTTGATATATATGCAGATGACATAGACCCTGCAATGCCT
341  A  T  E  D  N  G  L  F  D  I  Y  A  D  D  I  D  P  A  M  P  360
         1090      1100      1110      1020      1030      1040
     GTACCATCGCGTCCTACTACCTCCTCTGCAGTTTCTACATATTCGCCCACTATATCATCT
361  V  P  S  R  P  T  T  S  S  A  V  S  T  Y  S  P  T  I  S  S  380
         1150      1160      1170      1180      1190      1200
     GCCTCTTCCTATAGTAATGTAACGGTCCCTTTAACCTCCTCTTGGGATGTGCCTGTATAC
381  A  S  S  Y  S  N  V  T  V  P  L  T  S  S  W  D  V  P  V  Y  400
         1210      1220      1230      1240      1250      1260
     ACGGGTCCTGATATTACATTACCACCTACTACCTCTGTATGGCCCATTGTATCACCCACA
401  T  G  P  D  I  T  L  P  P  T  T  S  V  W  P  I  V  S  P  T  420
         1270      1280      1290      1300      1310      1320
     GCCCCTGCCTCTACACAGTATATTGGTATACATGGTACACATTATTATTTGTGGCCATTA
421  A  P  A  S  T  Q  Y  I  G  I  H  G  T  H  Y  Y  L  W  P  L  440
         1330      1340      1350      1360      1370      1380
     TATTATTTTATTCCTAAAAAGCGTAAACGTGTTCCCTATTTTTTTGCAGATGGCTTTGTG
441  Y  Y  F  I  P  K  K  R  K  R  V  P  Y  F  F  A  D  G  F  V  460

     GCGGCCTAG
461  A  A  *                                                      463
```

FIG. 3B

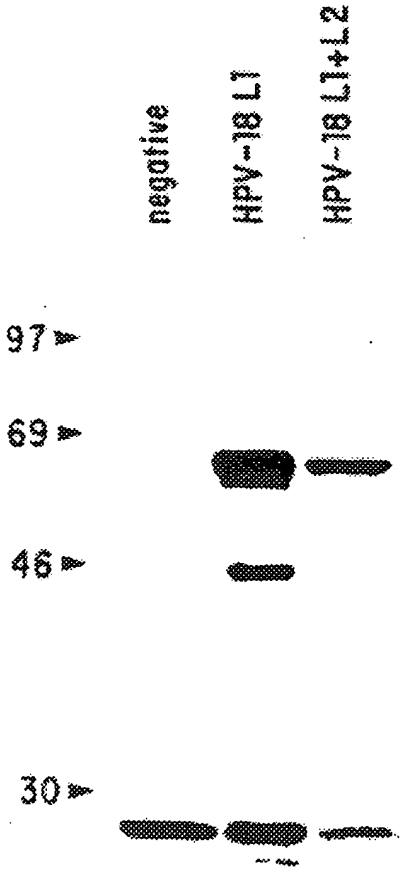
negative
HPV-18 L1
HPV-18 L1+L2
97
69
46
30

FIG.4

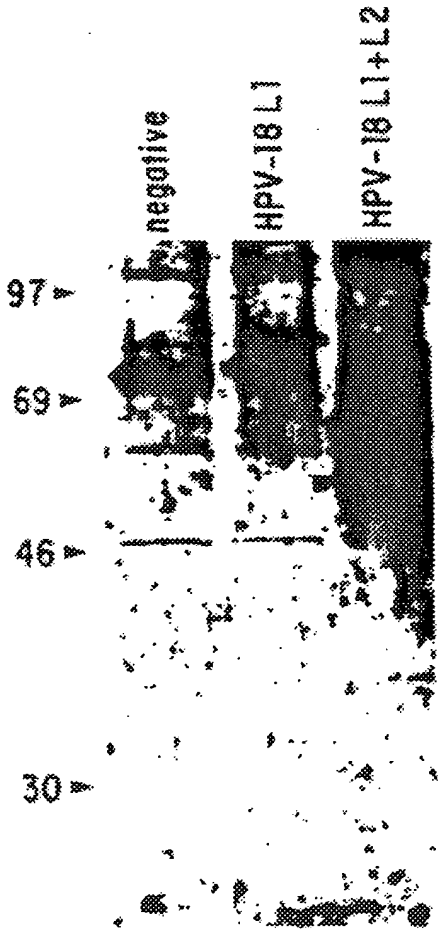
negative
HPV-18 L1
HPV-18 L1+L2
97
69
46
30

FIG.5

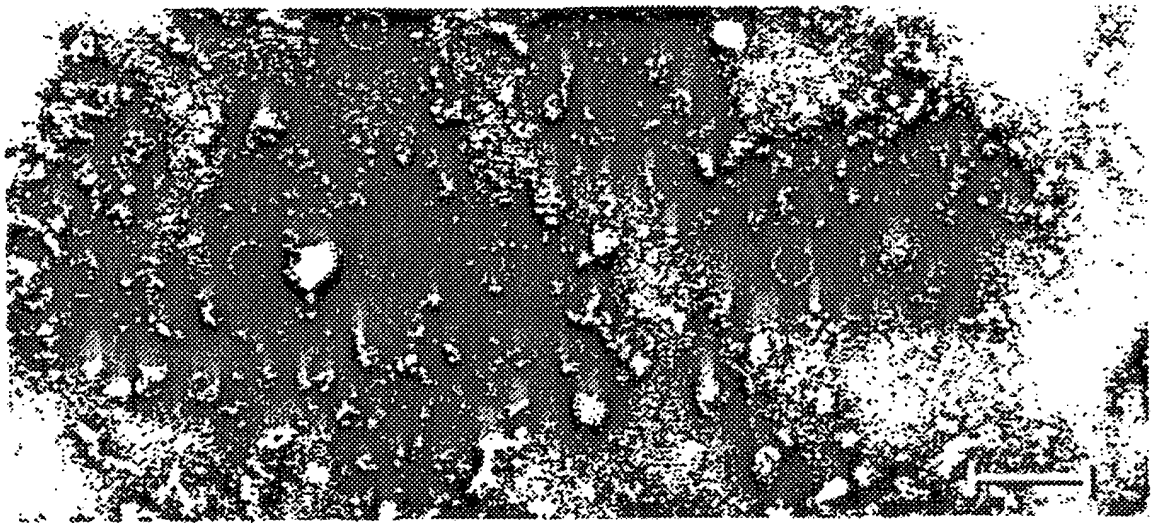

FIG.6

RECOMBINANT HUMAN PAPILLOMAVIRUS TYPE 18 VACCINE

FIELD OF THE INVENTION

The present invention is directed to DNA molecules encoding purified human papillomavirus type 18 and derivatives thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the HPV 18 L1 nucleotide (SEQ ID NO: 1) and deduced amino acid (SEQ ID NO:2) sequences.

FIG. 2 is a list of amino acid variations within the L1 protein of HPV 18.

FIG. 3 shows the HPV 18 L2 nucleotide (SEQ ID NO: 3) and deduced amino acid (SEQ ID NO: 4) sequences.

FIG. 4 shows an immunoblot of HPV 18 L1 protein expressed in yeast.

FIG. 5 shows an immunoblot of HPV 18 L2 protein expressed in yeast.

FIG. 6 is an electron micrograph of virus-like particles formed by HPV 18 L1 protein expressed in yeast.

BACKGROUND OF THE INVENTION

Papillomavirus (PV) infections occur in a variety of animals, including humans, sheep, dogs, cats, rabbits, monkeys, snakes and cows. Papillomaviruses infect epithelial cells, generally inducing benign epithelial or fibroepithelial tumors at the site of infection. PV are species specific infective agents; a human papillomavirus does not infect a nonhuman animal.

Papillomaviruses may be classified into distinct groups based on the host that they infect. Human papillomaviruses (HPV) are further classified into more than 70 types based on DNA sequence homology. PV types appear to be type-specific immunogens in that a neutralizing immunity to infection by one type of papillomavirus does not confer immunity against another type of papillomavirus.

In humans, different HPV types cause distinct diseases. HPV types 1, 2, 3, 4, 7, 10 and 26–29 cause benign warts in both normal and immunocompromised individuals. HPV types 5, 8, 9, 12, 14, 15, 17, 19–25, 36 and 46–50 cause flat lesions in immunocompromised individuals. HPV types 6, 11, 34, 39, 41–44 and 51–55 cause benign condylomata of the genital or respiratory mucosa. HPV types 16 and 18 cause epithelial dysplasia of the genital mucosa and are associated with the majority of in situ and invasive carcinomas of the cervix, vagina, vulva and anal canal.

Papillomaviruses are small (50–60 nm), nonenveloped, icosahedral DNA viruses that encode for up to eight early and two late genes. The open reading frames (ORFs) of the virus genomes are designated E1 to E7 and L1 and L2, where "E" denotes early and "L" denotes late. L1 and L2 code for virus capsid proteins. The early (E) genes are associated with functions such as viral replication and cellular transformation.

The L1 protein is the major capsid protein and has a molecular weight of 55–60 kDa. The L2 protein is a minor capsid protein which has a predicted molecular weight of 55–60 kDa and an apparent molecular weight of 75–100 kDa as determined by polyacrylamide gel electrophoresis. Immunological data suggest that most of the L2 protein is internal to the L1 protein within the viral capsomere. The L1 ORF is highly conserved among different papillomaviruses. The L2 proteins are less conserved among different papillomaviruses.

The L1 and L2 genes have been identified as good targets for immunoprophylaxis. Studies in the cottontail rabbit papillomavirus (CRPV) and bovine papillomavirus (BPV) systems have shown that immunizations with the L1 and L2 proteins expressed in bacteria or by using vaccinia vectors protected animals from viral infection. Expression of papillomavirus L1 genes in baculovirus expression systems or using vaccinia vectors resulted in the assembly of virus-like particles (VLP) which have been used to induce high-titered virus-neutralizing antibody responses that correlate with protection from viral challenge.

Following HPV type 16, HPV18 is the second most prevalent HPV type found in cervical carcinomas. HPV18 was detected in 5–20% of cervical cancer biopsies collected from various parts of the world (Ikenberg, H. 1990. Human papillomavirus DNA in invasive genital carcinomas. In *Genital Papillomavirus Infections*, G. Gross et al., eds. p. 85–112). There appears to be a geographic dependence of infection with HPV 18 since tumor biopsies from African and South American women harbor HPV 18 more frequently than similar biopsies from European and North American women. The underlying reasons for these geographic differences are not known. The development of a vaccine against HPV 18 infection becomes extremely relevant since HPV 18 is also associated with more aggressively growing cancers and is rarely found in the milder precursor lesions, CIN I-II.

SUMMARY OF THE INVENTION

The present invention is directed to DNA molecules encoding purified human papillomavirus type 18 (HPV type 18; HPV 18) and uses of the DNA molecules.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to DNA molecules encoding purified human papillomavirus type 18 (HPV type 18; HPV 18) and derivatives thereof. Such derivatives include but are not limited to peptides and proteins encoded by the DNA, antibodies to the DNA or antibodies to the proteins encoded by the DNA, vaccines comprising the DNA or vaccines comprising proteins encoded by the DNA, immunological compositions comprising the DNA or the proteins encoded by the DNA, kits containing the DNA or RNA derived from the DNA or proteins encoded by the DNA.

Pharmaceutically useful compositions comprising the DNA or proteins encoded by the DNA may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the DNA or protein or VLP. Such compositions may contain DNA or proteins or VLP derived from more than one type of HPV.

Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose PV infections. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration. Generally, the compositions will be administered in dosages ranging from about 1 μg to about 1 mg.

The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral, mucosal, intravenous and intramuscular.

The vaccines of the invention comprise DNA, RNA or proteins encoded by the DNA that contain the antigenic determinants necessary to induce the formation of neutralizing antibodies in the host. Such vaccines are also safe enough to be administered without danger of clinical infection; do not have toxic side effects; can be administered by an effective route; are stable; and are compatible with vaccine carriers.

The vaccines may be administered by a variety of routes, such as orally, parenterally, subcutaneously, mucosally, intravenously or intramuscularly. The dosage administered may vary with the condition, sex, weight, and age of the individual; the route of administration; and the type PV of the vaccine. The vaccine may be used in dosage forms such as capsules, suspensions, elixirs, or liquid solutions. The vaccine may be formulated with an immunologically acceptable carrier.

The vaccines are administered in therapeutically effective amounts, that is, in amounts sufficient to generate a immunologically protective response. The therapeutically effective amount may vary according to the type of PV. The vaccine may be administered in single or multiple doses.

The DNA and DNA derivatives of the present invention may be used in the formulation of immunogenic compositions. Such compositions, when introduced into a suitable host, are capable of inducing an immune response in the host.

The DNA or its derivatives may be used to generate antibodies. The term "antibody" as used herein includes both polyclonal and monoclonal antibodies, as well as fragments thereof, such as, Fv, Fab and F(ab)2 fragments that are capable of binding antigen or hapten.

The DNA and DNA derivatives of the present invention may be used to serotype HPV infection and HPV screening. The DNA, recombinant proteins, VLP and antibodies lend themselves to the formulation of kits suitable for the detection and serotyping of HPV. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as HPV 18 DNA, recombinant HPV protein or VLP or anti-HPV antibodies suitable for detecting a variety of HPV types. The carrier may also contain means for detection such as labeled antigen or enzyme substrates or the like.

The DNA and derived proteins therefrom are also useful as molecular weight and molecular size markers.

Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and therefore, the amino acid sequence can be encoded by any of a set of similar DNA oligonucleotides. Only one member of the set will be identical to the HPV 18 sequence but will be capable of hybridizing to HPV 18 DNA even in the presence of DNA oligonucleotides with mismatches under appropriate conditions. Under alternate conditions, the mismatched DNA oligonucleotides may still hybridize to the HPV 18 DNA to permit identification and isolation of HPV18 encoding DNA.

The purified HPV 18 DNA of the invention or fragments thereof may be used to isolate and purify homologues and fragments of HPV 18 from other sources. To accomplish this, the first HPV18 DNA may be mixed with a sample containing DNA encoding homologues of HPV 18 under appropriate hybridization conditions. The hybridized DNA complex may be isolated and the DNA encoding the homologous DNA may be purified therefrom.

It is known that there is a substantial amount of redundancy in the various codons which code for specific amino acids. Therefore, this invention is also directed to those DNA sequences which contain alternative codons which code for the eventual translation of the identical amino acid. For purposes of this specification, a sequence bearing one or more replaced codons will be defined as a degenerate variation. Also included within the scope of this invention are mutations either in the DNA sequence or the translated protein which do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in functionality of the polypeptide.

It is known that DNA sequences coding for a peptide may be altered so as to code for a peptide having properties that are different than those of the naturally-occurring peptide. Methods of altering the DNA sequences include, but are not limited to site-directed mutagenesis.

As used herein, a "functional derivative" of HPV 18 is a compound that possesses a biological activity (either functional or structural) that is substantially similar to the biological activity of HPV 18. The term "functional derivatives" is intended to include the "fragments," "variants," "degenerate variants," "analogs" and "homologues" or to "chemical derivatives" of HPV 18. The term "fragment" is meant to refer to any polypeptide subset of HPV 18 . The term "variant" is meant to refer to a molecule substantially similar in structure and function to either the entire HPV 18 molecule or to a fragment thereof. A molecule is "substantially similar" to HPV 18 if both molecules have substantially similar structures or if both molecules possess similar biological activity. Therefore, if the two molecules possess substantially similar activity, they are considered to be variants even if the structure of one of the molecules is not found in the other or even if the two amino acid sequences are not identical.

The term "analog" refers to a molecule substantially similar in function to either the entire HPV18 molecule or to a fragment thereof.

A variety of procedures may be used to molecularly clone HPV 18 DNA. These methods include, but are not limited to, direct functional expression of the HPV 18 genes following the construction of a HPV 18-containing cDNA or genomic DNA library in an appropriate expression vector system. Another method is to screen HPV 18-containing cDNA or genomic DNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled oligonucleotide probe designed from the amino acid sequence of the HPV 18. An additional method consists of screening a HPV 18-containing cDNA or genomic DNA library constructed in a bacteriophage or plasmid shuttle vector with a partial DNA encoding the HPV 18. This partial DNA is obtained by the specific polymerase chain reaction (PCR) amplification of HPV 18 DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence of purified HPV18. Another method is to isolate RNA from HPV 18-producing cells and translate the RNA into protein via an in vitro or an in vivo translation system. The translation of the RNA into a peptide or a protein will result in the production of at least a portion of HPV 18 protein which can be identified by, for example, the activity of HPV 18 protein or by immunological reactivity with an anti-HPV18 antibody. In this method, pools of RNA isolated from HPV 18-producing cells can be analyzed for the presence of an RNA which encodes at least a portion of the HPV 18. Further fractionation of the RNA pool can be done to purify the HPV 18 RNA from non-HPV 18 RNA. The peptide or protein produced by this method may be analyzed to provide

amino acid sequences which in turn are used to provide primers for production of HPV 18 cDNA, or the RNA used for translation can be analyzed to provide nucleotide sequences encoding HPV 18 and produce probes for the screening of a HPV 18 cDNA library. These methods are known in the art and can be found in, for example, Sambrook, J., Fritsch, E. F., Maniatis, T. in Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989.

It is apparent that other types of libraries, as well as libraries constructed from other cells or cell types, may be useful for isolating HPV 18-encoding DNA. Other types of libraries include, but are not limited to, cDNA libraries derived from other cells or cell lines containing HPV type 18 and genomic DNA libraries.

Preparation of cDNA libraries can be performed by a variety of techniques. cDNA library construction techniques can be found in Sambrook, J., et al., supra. It is apparent that DNA encoding HPV 18 may also be isolated from a suitable genomic DNA library. Construction of genomic DNA libraries can be performed by a variety of techniques. Genomic DNA library construction techniques can be found in Sambrook, J., et al. supra.

The cloned HPV 18 DNA or fragments thereof obtained through the methods described herein may be recombinantly expressed by molecular cloning into an expression vector containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant HPV 18. Techniques for such manipulations are fully described in Sambrook, J., et al., supra, and are known in the art.

Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned copies of genes and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic genes in a variety of hosts such as bacteria, bluegreen algae, plant cells, insect cells, fungal cells and animal cells. Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells or bacteria-fungal cells or bacteria-invertebrate cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses.

A variety of mammalian expression vectors may be used to express HPV 18 DNA or fragments thereof in mammalian cells. Commercially available mammalian expression vectors which may be suitable for recombinant HPV 18 expression, include but are not limited to, pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1 (8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and λZD35 (ATCC 37565).

A variety of bacterial expression vectors may be used to express HPV 18 DNA or fragments thereof in bacterial cells. Commercially available bacterial expression vectors which may be suitable include, but are not limited to pET11a (Novagen), lambda gt11 (Invitrogen), pcDNAII (Invitrogen), pKK223-3 (Pharmacia).

A variety of fungal cell expression vectors may be used to express HPV 18 or fragments thereof in fungal cells. Commercially available fungal cell expression vectors which may be suitable include but are not limited to pYES2 (Invitrogen), Pichia expression vector (Invitrogen), and Hansenula expression (Rhein Biotech, Dusseldorf, Germany).

A variety of insect cell expression vectors may be used to express HPV 18 DNA or fragments thereof in insect cells. Commercially available insect cell expression vectors which may be suitable include but are not limited to pBlue Bac III (Invitrogen) and pAcUW51 (PharMingen, Inc.).

An expression vector containing DNA encoding HPV 18 or fragments thereof may be used for expression of HPV 18 proteins or fragments of HPV 18 proteins in a cell, tissues, organs, or animals (including humans). Host cells may be prokaryotic or eukaryotic, including but not limited to bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to Drosophila and silkworm derived cell lines. Cell lines derived from mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L–M(TK–) (ATCC CCL 1.3), L cells L–M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, lipofection, protoplast fusion, and electroporation. The expression vector-containing cells are clonally propagated and individually analyzed to determine whether they produce HPV 18 protein. Identification of HPV 18 expressing host cell clones may be done by several means, including but not limited to immunological reactivity with anti-HPV 18 antibodies, and the presence of host cell-associated HPV18 activity, such as HPV 18-specific ligand binding or signal transduction defined as a response mediated by the interaction of HPV 18-specific ligands with the expressed HPV 18 proteins.

Expression of HPV DNA fragments may also be performed using in vitro produced synthetic mRNA or native mRNA. Synthetic mRNA or mRNA isolated from HPV18 producing cells can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

Following expression of HPV 18 protein(s) in a host cell, HPV 18 protein may be recovered to provide HPV 18 in purified form. Several HPV 18 purification procedures are available and suitable for use. As described herein, recombinant HPV 18 protein may be purified from cell lysates and extracts by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography.

In addition, recombinant HPV 18 may be separated from other cellular proteins by use of an immunoaffinity column

made with monoclonal or polyclonal antibodies specific for full length nascent HPV 18, or polypeptide fragments of HPV 18. Monoclonal and polygonal antibodies may be prepared according to a variety of methods known in the art. Monoclonal or monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for HPV 18. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope.

It is apparent that the methods for producing monospecific antibodies may be utilized to produce antibodies specific for HPV 18 polypeptide fragments, or full-length nascent HPV 18 polypeptide. Specifically, it is apparent that monospecific antibodies may be generated which are specific for the fully functional HPV 18 or fragments thereof.

The present invention is also directed toward methods for screening for compounds which modulate the expression of DNA or RNA encoding HPV 18 as well as the function(s) of HPV 18 protein(s) in vivo. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding HPV 18, or the function of HPV 18 protein. Compounds that modulate the expression of DNA or RNA encoding HPV 18 or the function of HPV 18 protein may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

Kits containing HPV 18 DNA, fragments of HPV 18 DNA, antibodies to HPV 18 DNA or HPV 18 protein, HPV 18 RNA or HPV 18 protein may be prepared. Such kits are used to detect DNA which hybridizes to HPV 18 DNA or to detect the presence of HPV 18 protein(s) or peptide fragments in a sample. Such characterization is useful for a variety of purposes including but not limited to forensic analyses and epidemiological studies.

Nucleotide sequences that are complementary to the HPV 18 encoding DNA sequence may be synthesized for antisense therapy. These antisense molecules may be DNA, stable derivatives of DNA such as phosphorothioates or methylphosphonates, RNA, stable derivatives of RNA such as 2'-O-alkylRNA, or other HPV 18 antisense oligonucleotide mimetics. HPV 18 antisense molecules may be introduced into cells by microinjection, liposome encapsulation or by expression from vectors harboring the antisense sequence. HPV 18 antisense therapy may be particularly useful for the treatment of diseases where it is beneficial to reduce HPV 18 activity.

The term "chemical derivative" describes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a variety of texts, such as Remington's Pharmaceutical Sciences.

Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages defined by routine testing in order to obtain optimal inhibition of the HPV 18 or its activity while minimizing any potential toxicity. In addition, co-administration or sequential administration of other agents may be desirable.

Advantageously, compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in several divided doses. Furthermore, compounds for the present invention may be administered via a variety of routes including but not limited to intranasally, orally, transdermally or by suppository.

For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times.

The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound thereof employed. A physician of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

In the methods of the present invention, the compounds herein described in detail can form the active ingredient, and are typically administered in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrup, suppositories, gels and the like, and consistent with conventional pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include without limitation, starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include, without limitation, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like.

For liquid forms the active drug component can be combined in suitably flavored suspending or dispersing agents such as the synthetic and natural gums, for example, tragacanth, acacia, methyl-cellulose and the like. Other dispersing agents which may be employed include glycerin and the like. For parenteral administration, sterile suspensions and solutions are desired. Isotonic preparations which generally contain suitable preservatives are employed when intravenous administration is desired.

Topical preparations containing the active drug component can be admixed with a variety of carrier materials well known in the art, such as, e.g., alcohols, aloe vera gel, allantoin, glycerine, vitamin A and E oils, mineral oil, PPG2 myristyl propionate, and the like, to form, e.g., alcoholic solutions, topical cleansers, cleansing creams, skin gels, skin lotions, and shampoos in cream or gel formulations.

The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Compounds of the present invention may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinyl-pyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamidephenol, polyhydroxy-ethylaspartamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

The following examples illustrate the present invention without, however, limiting the same thereto.

EXAMPLE 1

Cloning of HPV 18 genome

Total genomic DNA was prepared from the human cervical carcinoma-derived cell line, SW756 (Freedman, R. S., et al., 1982, In Vitro, Vol 18, pages 719–726) by standard techniques. The DNA was digested with EcoR1 and electrophoresed through a 0.8% low-melting temperature, agarose preparative gel. A gel slice was excised corresponding to DNA fragments approximately 12 kbp in length. The agarose was digested using Agarase™ enzyme (Boehringer Mannheim, Inc.) and the size-fractionated DNA was precipitated, dephosphorylated and ligated with EcoR1 digested lambda EMBL4 arms (Stratagene, Inc.). The lambda library was packaged using Gigapack II Gold packaging extract (Stratagene, Inc.). HPV 18-positive clones were identified using a 700 bp, HPV 18 L1 DNA probe that was generated by polymerase chain reaction (PCR) using SW756 DNA as template and oligonucleotide primers that were designed based on the published HPV18 L1 DNA sequence (Cole and Danos, 1987, *J. Mol. Biol.*, Vol. 193:599–608; Genbank Accession #X05015). A HPV 18-positive, lambda clone was isolated that contained a 12 kbp EcoR1 fragment insert and was designated as #187-1.

EXAMPLE 2

Construction of Yeast Expression Vectors

The HPV 18 L1 open reading frame (ORF) was amplified by PCR using clone #187-1 as template, Vent polymerase™ (New England Biolabs, Inc.), 10 cycles of amplification (94° C., 1 min; 50° C., 1 min; 72° C. 2 min) and the following oligonucleotide primers which contain flanking BglII sites (underlined): sense primer,

5'-GAAGATCTCACAAAACAAAATGGCTTGTGG CGGCCTAGTG-3',(SEQ ID NO:5)

antisense primer,

5'-GAAGATCTFTACTTCCTGGCACGTAC ACGCACACGC-3' (SEQ ID NO: 6).

The sense primer introduces a yeast non-translated leader sequence (Kniskern, et al., 1986, Gene, Vol. 46:135–141) immediately upstream to the HPV 18 L1 initiating methionine codon (highlighted in bold print). The 1.5 kbp L1 PCR product was digested with BglII and gel purified.

The pGAL1–10 yeast expression vector was constructed by isolating a 1.4 kbp SphI fragment from a pUC 18/ bidirectional GAL promoter plasmid which contains the *Saccharomyces cerevisiae* divergent GAL1–GAL10 promoters from the plasmid pBM272 (provided by Mark Johnston, Washington University, St. Louis, Mo.). The divergent promoters are flanked on each side by a copy of the yeast ADH1 transcriptional terminator, a BamHI cloning site located between the GAL1 promoter and the first copy of the ADH1 transcriptional terminator and a SmaI cloning site located between the GAL10 promoter and the second copy of the ADH1 transcriptional terminator. A yeast shuttle vector consisting of pBR322, the yeast LEU2d gene, and the yeast 2u plasmid (gift of Benjamin Hall, University of Washington, Seattle, Wash.A) was digested with SphI and ligated with the 1.4 kbp SphI divergent GAL promoter fragment resulting in pGAL1–10. pGAL1–10 was linearized with BamHI which cuts between the GAL1 promoter and the ADH1 transcription terminator. The BamHI digested vector and the BglII digested HPV 18 L1 PCR fragment were ligated and used to transform *E. coli* DH5 cells (Gibco BRL, Inc.). A pGAL1–10 plasmid was isolated which contains the HPV 18 L1 gene and was designated p191–6.

A yeast expression vector that co-expresses both the HPV 18 L1 and L2 genes was constructed. Plasmid p191–6 (pGAL1–10 + HPV 18 L1) was digested with SmaI which cuts between the GAL 10 promoter and the ADH1 transcription terminator. The 1.4 kbp HPV 18 L2 gene was amplified by PCR as described above using the following oligonucleotide primers which contain flanking SmaI sites (underlined): sense primer,

5'-TCCCCCGGGCACAAAACAAAATG GTATCCCACCGTGCCGCACGAC-3' (SEQ ID NO:7)

antisense primer,

5'-TCCCCCGGGCTAGGCCGCCACAAAGCCA TCTGC-3' (SEQ ID NO:8) .

The sense primer introduces a yeast non-translated leader sequence (Kniskern et al., 1986, supra) immediately upstream to the HPV 18 L2 initiating methionine codon (highlighted in bold print). The PCR fragment was digested with SmaI, gel purified and ligated with the SmaI digested p191-6 plasmid. A pGAL1–10 plasmid containing both the HPV 18 L1 and L2 genes was isolated and designated, p195-11.

EXAMPLE 3

Typing of Clinical Samples

Cervical biopsy samples were collected at the Veterans Administration Medical Center in Indianapolis, Ind. (courtesy of Dr. Darron Brown) and at the Albert Einstein Medical Center in Philadelphia, Pa. (courtesy of Dr. Joan Adler) and were frozen at -20° C. DNA was isolated as described by Brown et al., 1993 (Brown, D. et al., 1993, J. clin. Microbiol., Vol. 31:2667–2673). Briefly, clinical specimens were processed with a Braun mikro-dismembrator II (B. Braun Instruments, Melsungen, Germany) and solubilized in buffer containing 10 mM EDTA and 0.6% (w/v) sodium dodecyl sulfate (SDS). Samples were adjusted to 20 mM Tris pH 7.4 and protein was digested with 50 mcg/mL Proteinase K in the presence of 0.1 mcg/mL RNase A followed by extraction with phenol/chloroform/isoamyl alcohol. DNA was ethanol precipitated and quantified by spectrophotometry.

The DNA samples were screened for the presence of HPV 18 by PCR and Southern blot analyses. A 256 bp segment of the HPV 18 L1 ORF was amplified by PCR using the following oligonucleotide primers: sense primer,

5'-CAATCCTTATATATTAAAGGCACAGGTATG-3', antisense primer (SEQ ID NO:9),

5'-CATCATATTGCCCAGGTACAGGAGACTGTG-3' (SEQ ID NO:10).

The PCR conditions were according to the manufacturer's recommendations for the AmpliTaq™ DNA Polymerase/ GeneAmp™ kit (Perkin Elmer Corp.) except that 0.5 μl of clinical sample DNA was used as template and 10 pmoles of each primer, 2 mM dNTPs and 2.0 mM $MgCl_2$ were in the final reaction mixture. A 2 min, 94° C. denaturation step was followed by 40 cycles of amplification (94° C., 1 min; 45° C. 1 min; 72° C. 1 min.).

The PCR products were electrophoresed through a 3.0% agarose gel, blotted onto nylon membranes and hybridized with a ^{32}P-labeled HPV 18 L1-specific oligonucleotide probe.

EXAMPLE 4

DNA Sequencing of L1 and L2 genes

The HPV18 L1 and L2 genes in clones #187–1, p191–6 and p195–11 were sequenced using the PRIZM Sequencing kit and the automated DNA ABI Sequencer #373A (Applied Biosystems). To obtain a consensus HPV 18 sequence, portions of the L1 gene DNA were amplified by PCR from human clinical isolates, sequenced and compared to the claimed and published sequences. A 256 bp fragment (nucleotides 817–1072) was amplified from each clinical DNA isolate for this purpose using the oligonucleotides and heating cycles described in Example 3. The following primers,

5'-GAAGATCTCACAAAACAAA ATGGCTTTGTGGCGGCCTAGTG-3' (SEQ ID NO: 11) and 5'- CCTAACGTCCTCAGAAACATTAGAC-3' (SEQ ID NO:12) were used to amplify an amino-terminal 432 bp portion of L1 DNA (nucleotides 1–431) using the heating cycles described in Example 3. Both PCR products were ligated separately with plasmid pCRII (Invitrogen Corp.) using the reagents and procedures recommended by the manufacturer. Plasmid DNA was isolated from the transformants and those containing EcoRI inserts were sequenced.

EXAMPLE 5

Analysis of DNA and Deduced Amino Acid Sequences

The nucleotide and deduced amino acid (aa) sequences of the claimed HPV18 L1 are shown in FIG. 1. The DNA sequence was derived from a consensus of clones #187-1, p191-6 and p195-11. A comparison of the claimed HPV 18 L1 nucleotide sequence with the published HPV 18 L1 sequence (Genbank Accession #X05015) identified 20 bp changes out of 1524 bps. Five of the nucleotide changes (C to G at position 89, C to A at 263, C to G at 848, G to A at 967 and C to G at 1013) result in amino acid substitutions. The five residue differences from published are P to R at aa positions 30, 283 and 338, T to N at aa 88 and V to I at aa 323 (FIG. 2). Positions 88 and 323 represent conservative changes while the three P to R changes may substantially alter the physical properties of the expressed L1 protein.

A comparison of the amino acid sequences derived from clinical isolates (numbers 354, 556, 755, 697, 795 and 23) with the claimed sequence and the published sequence is shown in FIG. 2. There are four locations where the clinical isolates and the claimed sequence differ from the published sequence. Positions 30, 283 and 338 encode arginine (R) in all the isolates found to date, including the claimed sequence. This is in sharp contrast to the published sequence which has prolines (P) at each of these locations. Furthermore, position 88 is an asparagine (N) in the isolates and the claimed sequence but is a threonine (T) in the published sequence. The last difference, position 323, was found to be a valine (V) in many of the clinical isolates and the published strain versus an isoleucine (I) in the claimed sequence and one of the isolates (#23). The conclusion is that the claimed sequence reflects the predominant viral sequences that are associated with clinical infections and the absence of isolates containing any of the position 30, 283 or 338 prolines of the published sequence suggests that the published clone is either an artefact or an inconsequential subtype.

The nucleotide and deduced aa sequences of HPV 18 L2 were derived from a consensus sequence of clones #187–1 and p195–11 and are shown in FIG. 3. A comparison of the L2 nucleotide sequence with the published HPV 18 sequence (Genbank Accession #X05015) identified 40 bp changes out of 1389 bps. The bp differences result in 14 changes at the aa level: P to S at aa 29, P to N at aa 33, A to S at aa 177, D to E at aa 266, D to N at aa 270, D to G at aa 346, M to I at 355, V to M at aa 359, S to P at aa 365, F to S at aa 369, F to V at aa 371, F to S at aa 372, K to T at aa 373 and S to P at aa 409.

EXAMPLE 6

Generation of HPV 18 L2 Antiserum

HPV 18 L2 specific antibodies were prepared in goats using a trpE-HPV 18 L2 fusion protein expressed in *E. coli*. The full-length L2 ORF was amplified by PCR using oligonucleotide primers providing HindIII and BamHI sites flanking the 5'- and 3'- ends, respectively. The L2 fragment was inserted into the HindIII-BamHI digested, pATH23 expression plasmid (Koerner at al., 1991, Meth. Enzymol. Vol. 194:477–490). The fusion protein was expressed in *E. coli* RR1 cells (Gibco BRL, Inc.) after induction with 3-b-indoleacrylic acid. The insoluble fraction was analyzed by SDS-PAGE followed by staining with Coomassie Blue. The trpE-L2 fusion protein accounted for the major portion of the *E. coli* insoluble fraction. Goats were immunized with the trpE-L2 fusion protein according to the standard protocol of Pocono Rabbit Farm and Laboratory, Inc. for fusion protein antigens (Protein Rabbit Farm, Canadensis, Pa.).

EXAMPLE 7

Preparation of Yeast Strain U9

Saccharomyces cerevisiae strain 2150-2-3 (MATalpha, leu2-O4, adel, cir°) was obtained from Dr. Leland Hartwell (University of Washington, Seattle, Wash.). Cells of strain 2150-2-3 were propagated overnight at 30° C. in 5 mL of YEHD medium (Carty et al., *J. Ind Micro* 2 (1987) 117–121). The cells were washed 3 times in sterile, distilled water, resuspended in 2 mL of sterile distilled water, and 0.1 mL of cell suspension was plated onto each of six 5-fluoro-orotic acid (FOA) plates in order to select for ura3 mutants (Cold Spring Harbor Laboratory Manual for Yeast Genetics). The plates were incubated at 30° C. The medium contained per 250 mL distilled water: 3.5 g, Difco Yeast Nitrogen Base without amino acids and ammonium sulfate; 0.5 g 5- Fluoro-orotic acid; 25 mg Uracil; and 10.0 g Dextrose.

The medium was sterilized by filtration through 0.2 μm membranes and then mixed with 250 mL of 4% Bacto-Agar (Difco) maintained at 50° C., 10 mL of a 1.2 mg/mL solution of adenine, and 5 mL of L-leucine solution (180 mg/ 50 mL). The resulting medium was dispensed at 20 mL per petri dish.

After 5 days of incubation, numerous colonies had appeared. Single colonies were isolated by restreaking colonies from the initial FOA plates onto fresh FOA plates which

were then incubated at 30° C. A number of colonies from the second set of FOA plates were tested for the presence of the ura3 mutation by replica-plating onto both YEHD plates and uracil-minus plates. The desired result was good growth on YEHD and no growth on uracil-minus medium. One isolate (U9) was obtained which showed these properties. It was stored as a frozen glycerol stock (strain #325) at -70° C. for later use.

EXAMPLE 8

Preparation of a Vector for disruption of the Yeast MNN9 gene

In order to prepare a vector for disruption of the MNN9 gene, it was necessary to first clone the MNN9 gene from *S. cerevisiae* genomic DNA. This was accomplished by standard Polymerase Chain Reaction (PCR) technology. A 5 ' sense primer and 3 ' antisense primer for PCR of the full-length MNN9 coding sequence were designed based on the published sequence for the yeast MNN9 gene (Zymogenetics: EPO Patent Application No. 88117834.7, Publication No. 0-314-096-A2). The following oligodeoxynucleotide primers containing flanking HindIII sites (underlined) were used:

sense primer: 5'-CTT AAA GCT TAT GTC ACT TTC TCT TGT ATC G-3' (SEQ ID NO:13)

antisense primer: 5'-TGA TAA GCT TGC TCA ATG GTT CTC TTC CTC-3' (SEQ ID NO: 14) .

The initiating methionine codon for the MNN9 gene is highlighted in bold print. The PCR was conducted using genomic DNA from *S. cerevisiae* strain JRY 188 as template, Taq DNA polymerase (Perkin Elmer) and 25 cycles of amplification (94° C. 1 min., 37° C. 2 min., 72° C. 3 min.). The resulting 1.2 kbp PCR fragment was digested with HindIII, gel-purified, and ligated with HindIII-digested, alkaline-phosphatase treated pUC13 (Pharmacia). The resulting plasmid was designated p 1183.

In order to disrupt the MNN9 gene with the yeast URA3 gene, the plasmid pBR322-URA3 (which contains the 1.1 Kbp HindIII fragment encoding the *S. cerevisiae* URA3 gene subcloned into the HindIII site of pBR322) was digested with HindIII and the 1.1 kbp DNA fragment bearing the functional URA3 gene was gel-purified, made blunt-ended with T4 DNA polymerase, and then ligated with PmlI-digested plasmid p1183 (PmlI cuts within the MNN9 coding sequence). The resulting plasmid p1199 contains a disruption of the MNN9 gene by the functional URA3 gene.

EXAMPLE 9

Construction of U9-derivative strain 1372 containing disruption of MNN9 gene

For disruption of the MNN9 gene in strain U9 (#325), 30 μg of plasmid p1199 were digested with HindIII to create a linear mnn9::URA3 disruption cassette. Cells of strain 325 were transformed with the HindIII-digested p1199 DNA by the spheroplast method (Hinnen et al., 1978, Proc. Natl. Acad. Sci. USA 75:1929–1933) and transformants were selected on a synthetic agar medium lacking uracil and containing 1.0M sorbitol. The synthetic medium contained, per liter of distilled water: Agar, 20 g; Yeast nitrogen base w/o amino acids, 6.7 g; Adenine, 0.04 g; L-tyrosine, 0.05 g; Sorbitol, 182 g; Glucose, 20 g; and Leucine Minus Solution #2, 10 ml. Leucine Minus Solution #2 contains per liter of distilled water: L-arginine, 2 g; L-histidine, 1 g; L-Leucine, 6 g; L-Isoleucine, 6 g; L-lysine, 4 g; L-methionine, 1 g; L-phenylalanine, 6 g; L-threonine, 6 g; L-tryptophan, 4 g.

The plates were incubated at 30° C. for five days at which time numerous colonies had appeared. Chromosomal DNA preparations were made from 10 colonies and then digested with EcoRI plus HindIII. The DNA digests were then evaluated by Southern blots (J. Sambrook et al., *Molecular Cloning: A Laboratory Manual,* 2nd edition, Cold Spring Harbor Laboratory Press, 1989) using the 1.2 kbp HindIII fragment bearing the MNN9 gene (isolated from plasmid p1199) as a probe. An isolate was identified (strain #1372) which showed the expected DNA band shifts on the Southern blot as well as the extreme clumpiness typically shown by mnn9 mutants.

EXAMPLE 10

Construction of a Vector for Disruption of Yeast HIS3 Gene

In order to construct a disruption cassette in which the *S. cerevisiae* HIS3 gene is disrupted by the URA3 gene, the plasmid YEp6 (K. Struhl et al., 1979, Proc. Natl. Acad. Sci., USA 76:1035) was digested with BamHI and the 1.7 kbp BamHI fragment bearing the HIS3 gene was gel-purified, made blunt-ended with T4 DNA polymerase, and ligated with pUC 18 which had been previously digested with BamHI and treated with T4 DNA polymerase. The resulting plasmid (designated p1501 or pUC18-HIS3) was digested with NheI (which cuts in the HIS3 coding sequence), and the vector fragment was gel-purified, made blunt-ended with T4 DNA polymerase, and then treated with calf intestine alkaline phosphatase. The URA3 gene was isolated from the plasmid pBR322-URA3 by digestion with HindIII and the 1.1 kbp fragment bearing the URA3 gene was gel-purified, made blunt-ended with T4 DNA polymerase, and ligated with the above pUC18-HIS3 NheI fragment. The resulting plasmid (designated pUC18-his3::URA3 or p1505) contains a disruption cassette in which the yeast HIS3 gene is disrupted by the functional URA3 gene.

EXAMPLE 11

Construction of Vector for Disruption of Yeast PRB1 Gene by the HIS3 Gene

Plasmid FP8ΔH bearing the *S. cerevisiae* PRB1 gene was provided by Dr. E. Jones of Carnegie-Mellon Univ. (C. M. Moehle et al., 1987, *Genetics* 115:255–263). It was digested with HindIII plus XhoI and the 3.2 kbp DNA fragment bearing the PRBI gene was gel-purified and made blunt-ended by treatment with T4 DNA polymerase. The plasmid pUC18 was digested with BamHI, gel-purified and made blunt-ended by treatment with T4 DNA polymerase. The resulting vector fragment was ligated with the above PRBI gene fragment to yield the plasmid pUC18-PRB1. Plasmid YEp6, which contains the HIS3 gene, was digested with BamHI. The resulting 1.7 kbp BamHI fragment bearing the functional HIS3 gene was gel-purified and then made blunt-ended by treatment with T4 DNA polymerase. Plasmid pUC18-PRB18 was digested with EcoRV plus NcoI which cut within the PRB1 coding sequence and removes the protease B active site and flanking sequence. The 5.7 kbp EcoRV-NcoI fragment bearing the residual 5' and 3'- portions of the PRB1 coding sequence in pUC18 was gel-purified, made blunt-ended by treatment with T4 DNA polymerase, dephosphorylated with calf intestine alkaline phosphatase, and ligated with the blunt-ended HIS3 fragment described above. The resulting plasmid (designated pUC 18-prb1::HIS3, stock #1245) contains the functional HIS3 gene in place of the portion of the PRB1 gene which had been deleted above.

EXAMPLE 12

Construction of a U9-related Yeast Strain containing disruptions of both the MNN9 and PRB1 Genes

The U9-related strain 1372 which contains a MNN9 gene disruption was described in Example 9. Clonal isolates of

strain 1372 were passaged on FOA plates (as described in Example 7) to select ura3 mutants. A number of ura3 isolates of strain 1372 were obtained and one particular isolate (strain 12930-190-S1-1) was selected for subsequent disruption of the HIS3 gene. The pUC18-his3::URA3 gene disruption vector (p1505) was digested with XbaI plus EcoRI to generate a linear his3::URA3 disruption cassette and used for transformation of strain 12930-190-S1-1 by the lithium acetate method [*Methods in Enzymology*, 194:290 (1991)]. Ura transformants were selected on synthetic agar medium lacking uracil, restreaked for clonal isolates on the same medium, and then replica-plated onto medium lacking either uracil or histidine to screen for those isolates that were both Ura^{30} and His^{-}. One isolate (strain 12930- 230- 1) was selected for subsequent disruption of the PRB1 gene. The PRB1 gene disruption vector (pUC18-prb1::HIS3, stock #1245) was digested with SacI plus XbaI to generate a linear prb1::HIS3 disruption cassette and used for transformation of strain 12930-230-1 by the lithium acetate method. His^{+} transformants were selected on agar medium lacking histidine and restreaked on the same medium for clonal isolates. Genomic DNA was prepared from a number of the resulting His^{+}isolates, digested with EcoRI, and then electrophoresed on 0.8% agarose gels. Southern blot analyses were then performed using a radio-labeled 617 bp probe for the PRB1 gene which had been prepared by PCR using the following oligodeoxynucleotide primers:

5' TGG TCA TCC CAA ATC TTG AAA 3' (SEQ ID NO:15)

5' CAC CGT AGT GTT TGG AAG CGA 3' (SEQ ID NO:16)

Eleven isolates were obtained which showed the expected hybridization of the probe with a 2.44 kbp prb1 ::HIS3 DNA fragment. This was in contrast to hybridization of the probe with the 1.59 kbp fragment for the wild-type PRB1 gene. One of these isolates containing the desired prb1::HIS3 disruption was selected for further use and was designated strain #1558.

EXAMPLE 13

Expression of HPV 18 L1 and L2 in Yeast

Plasmids p191–6 (pGAL1-10 + HPV 18 L1) and p195-11 (pGAL1–10 + HPV 18 L1 +L2) were used to transform *S. cerevisiae* strain #1558 (MATa, leu2O4, prb1::HIS3, mnn9::URA3, adel, cir^{0}). Clonal isolates were grown at 30° C. in YEHD medium containing 2% galactose for 88 hours. After harvesting the cells, the cell pellets were broken with glass beads and cell lysates analyzed for the expression of HPV 18 L1 and/or HPV 18 L2 protein by immunoblot analysis. Samples containing 25 ug of total cellular protein were electrophoresed through 10% Tris-Glycine gels (Novex, Inc.) under denaturing conditions and electroblotted onto nitrocellulose filters. L1 protein was immunodetected using rabbit antiserum raised against a trpE-HPV 11 L1 fusion protein as primary antibody (Brown et al., 1994, Virology 201:46–54) and horseradish peroxidase (HRP)-linked donkey anti-rabbit IgG (Amersham, Inc.) as secondary antibody. The filters were processed using the chemiluminescent ECL™ Detection Kit (Amersham, Inc.). A 50–55 KDa L1 protein band was detected in both the L1 and L1 +L2 coexpressor yeast clones (strains 1725 and 1727, respectively) and not in the negative control (pGAL1–10 without L1 or L2 genes) (FIG. 4).

The HPV 18 L2 protein was detected by Western analysis using goat polyclonal antiserum raised against a trpE-HPV 18 L2 fusion protein as primary antibody followed by HRP-conjugated, rabbit anti-goat IgG (Kirkegaard and Perry Laboratories, Gaithersburg, Md.). The filters were processed as described above. The L2 protein was detected as a 75 kDa protein band in the L1+L2 coexpressor yeast clone (strain 1727) but not in either the negative control or the L1 expressor clone (FIG. 5).

EXAMPLE 14

Fermentation of HPV 18 L1 (strain 1725) and 18 L1+ΔL2 (strain 1727).

Surface growth of a plate culture of strains 1725 and 1727 was aseptically transferred to a leucine-free liquid medium containing (per L): 8.5 g Difco yeast nitrogen base without amino acids and ammonium sulfate; 0.2 g adenine; 0.2 g uracil; 10 g succinic acid; 5 g ammonium sulfate; 40 g glucose; 0.25 g L-tyrosine; 0.1 g L-arginine; 0.3 g L-isoleucine; 0.05 g L-methionine; 0.2 g L-tryptophan; 0.05 g L-histidine; 0.2 g L-lysine; 0.3 g L-phenylalanine; this medium was adjusted to pH 5.0–5.3 with NaOH prior to sterilization. After growth at 28° C., 250 rpm on a rotary shaker, frozen culture vials were prepared by adding sterile glycerol to a final concentration of 17% (w/v) prior to storage at −70° C. (1 mL per cryovial). Inocula were developed in the same medium (500 mL per 2-L flask) and were started by transferring the thawed contents of a frozen culture vial and incubating at 28° C., 250 rpm on a rotary shaker for 29 hr. Fermentations of each strain used a New Brunswick SF-116 fermentor with a working volume of 10 L after inoculation. The production medium contained (per L): 20 g Difco yeast extract; 10 g Sheffield HySoy peptone; 20 g glucose; 20 g galactose; 0.3 mL Union Carbide UCON LB-625 antifoam; the medium was adjusted to pH 5.3 prior to sterilization. The entire contents (500 mL) of the 2-L inoculum flask was transferred to the fermentor which was incubated at 28° C., 5 L air per min, 400 rpm, 3.5 psi pressure. Agitation was increased as needed to maintain dissolved oxygen levels of greater than 40% of saturation. Progress of the fermentation was monitored by off-line glucose measurements (Beckman Glucose 2 Analyzer) and on-line mass spectrometry (Perkin-Elmer 1200). After incubation for 66 hr, cell densities of 9.5 to 9.7 g dry cell weight per L were reached. The cultures were concentrated by hollow fiber filtration (Amicon H5MPO1–43 cartridge in an Amicon DC-10 filtration system) to ca. 2 L, diafiltered with 2 L phosphate-buffered saline, and concentrated further (to ca. 1 L) before dispensing into 500-mL centrifuge bottles. Cell pellets were collected by centrifugation at 8,000 rpm (Sorval GS-3 rotor) for 20 min at 4 ° C. After decanting the supernatant, the pellets (total 191 to 208 g wet cells) were stored at −70° C. until use.

EXAMPLE 15

Purification of Recombinant HPV Type 18 L1 Capsid Proteins

All steps performed at 4° C. unless noted.

Cells were stored frozen at −70° C. Frozen cells (wet weight =126 g) were thawed at 20°–23° C. and resuspended in 70 mL "Breaking Buffer" (20 mM sodium phosphate, pH 7.2, 100 mM NaCl). The protease inhibitors PMSF and pepstatin A were added to final concentrations of 2 mM and 1.7 μM, respectively. The cell slurry was broken at a pressure of approximately 16,000 psi by 4 passes in a M 110-Y Microfluidizer (Microfluidics Corp., Newton, Mass.). The broken cell slurry was centrifuged at 12,000×g for 40 min to remove cellular debris. The supernatant liquid containing L1 antigen was recovered.

The supernatant liquid was diluted 1:5 by addition of Buffer A (20 mM MOPS, pH 7.0) and applied to an anion exchange capture column (9.0 cm ID ×3.9 cm) of Fracto-

gel® EMD TMAE-650 (M) resin (EM Separations, Gibbstown, N.J.) equilibrated in Buffer A. Following a wash with Buffer A, the antigen was eluted with a gradient of 0–1.0M NaCl in Buffer A. Column fractions were assayed for total protein by the Bradford method. Fractions were then analyzed at equal total protein loadings by Western blotting and SDS-PAGE with silver stain detection.

TMAE fractions showing comparable purity and enrichment of L1 protein were pooled. The antigen was concentrated by ammonium sulfate fractionation. The solution was adjusted to 35% saturated ammonium sulfate by adding solid reagent while gently stirring over 10 min. The sample was placed on ice and precipitation allowed to proceed for 4 hours. The sample was centrifuged at 16,000×g for 45 min. The pellet was resuspended in 20.0 mL PBS (6.25 mM Na phosphate, pH 7.2, 150 mM NaCl).

The resuspended pellet was chromatographed on a size exclusion column (2.6 cm ID×89 cm) of Sephacryl 500 HR resin (Pharmacia, Piscataway, N.J.). Running buffer was PBS. Fractions were analyzed by western blotting and SDS-PAGE with silver stain detection. The purest fractions were pooled. The resulting pool was concentrated in a 50 mL stirred cell using 43 mm YM-100 flat-sheet membranes (Amicon, Beverly, Mass.) at a N_2 pressure of 4–6 psi.

Final product was analyzed by western blotting and SDS-PAGE with colloidal Coomassie detection. The L1 protein was estimated to be 50–60% homogeneous. The identity of L1 protein was confirmed by western blotting. The final product was aliquoted and stored at −70° C. This process resulted in a total of 12.5 mg protein.

Bradford Assay for Total Protein

Total protein was assayed using a commercially available Coomassie Plus® kit (Pierce, Rockford, Ill.). Samples were diluted to appropriate levels in Milli-Q-H_2O. Volumes required were 0.1 mL and 1.0 mL for the standard and microassay protocols, respectively. For both protocols, BSA (Pierce, Rockford, Ill.) was used to generate the standard curve. Assay was performed according to manufacturer's recommendations. Standard curves were plotted using CricketGraph® software on a Macintosh IIci computer.

SDS-PAGE and Western Blot Assays

All gels, buffers, and electrophoretic apparatus were obtained from Novex (San Diego, Calif.) and were run according to manufacturer's recommendations. Briefly, samples were diluted to equal protein concentrations in Milli-Q-H_2O and mixed 1:1 with sample incubation buffer containing 200 mM DTT. Samples were incubated 15 min at 100° C. and loaded onto pre-cast 12% Tris-glycine gels. The samples were electrophoresed at 125 V for 1 hr 45 min. Gels were developed using either silver staining by a variation of the method of Heukeshoven and Demick [Electrophoresis, 6 (1985) 103–112] or colloidal Coomassie staining using a commercially obtained kit (Integrated Separation Systems, Natick, Mass.).

For western blots, proteins were transferred to PVDF membranes at 25 V for 40 min. Membranes were washed with Milli-Q-H_2O and air-dried. Primary antibody was polyclonal rabbit antiserum raised against a TrpE-HPV11L1 fusion protein (gift of Dr. D. Brown). Previous experiments had shown this antiserum to cross react with HPV type 18 L1 on western blots. The antibody solution was prepared by dilution of antiserum in blotting buffer (5% non-fat milk in 6.25 mM Na phosphate, pH 7.2, 150 mM NaCl, 0.02% NaN_3). Incubation was for at least 1 hour at 20°–23° C. The blot was washed for 1 min each in three changes of PBS (6.25 mM Na phosphate, pH 7.2, 150 mM NaCl). Secondary antibody solution was prepared by diluting goat anti-rabbit IgG alkaline phosphatase-linked conjugate antiserum (Pierce, Rockford, Ill.) in blotting buffer. Incubation proceeded under the same conditions for at least 1 hour. Blots were washed as before and detected using a 1 step NBT/BCIP substrate (Pierce, Rockford, Ill.).

EXAMPLE 16

Electron Microscopic Studies

For EM analysis (Structure Probe, West Chester, Pa.), an aliquot of each sample was placed on 200-mesh carbon-coated copper grids. A drop of 2% phosphotungstic acid (PTA), pH 7.0 was placed on the grid for 20 seconds. The grids were allowed to air dry prior to transmission EM examination. All microscopy was done using a JEOL 100CX transmission electron microscope (JEOL USA, Inc.) at an accelerating voltage of 100 kV. The micrographs generated have a final magnification of 100,000×. Virus-like particles were observed in the 50–55 nm diameter size range in the yeast sample harboring the HPV18 L1 expression plasmid (FIG. 6). No VLP's were observed in the yeast control samples.

EXAMPLE 17

Sub-cloning of the HPV18 cDNA into expression vectors

The cDNA encoding HPV18 is sub-cloned into several vectors for expression of the HPV18 protein in transfected host cells and for in vitro transcription/translation. These vectors include pBluescript II SK+ (where expression is driven by T7 or T3 promoters) pcDNA I/Amp (where expression is driven by the cytomegalovirus (CMV) promoter), pSZ9016-1 (where expression is driven by the HIV long terminal repeat (LTR) promoter) and the baculovirus transfer vector pAcUW51 (PharMingen, Inc.) (where expression is driven by the polyhedrin (PH) promoter) for producing recombinant baculovirus containing the HPV18 encoding DNA sequence.

a) pBluescript II SK+:HPV18. The full length HPV18 cDNA clone is retrieved from lambda bacteriophage by limited Eco RI digestion and ligated into Eco RI-cut, CIP-treated pBluescript II SK+. Separate subclones are recovered in which the sense orientation of HPV18 followed either the T7 or T3 promoters.

b)pcDNA I/Amp:HPV18. To facilitate directional cloning, HPV18 is excised from a purified plasmid preparation of pBluescript II SK+:HPV18 in which the HPV18 DNA sequence is downstream of the T7 promoter using Eco RV and Xba. I. The resulting Eco RV, Xba I HPV18 fragment is purified and ligated into Eco RV-cut, Xba I-cut, CIP-treated pcDNA I/Amp such that the HPV18 encoding DNA is downstream of the CMV promoter.

c)pSZ9016-1:HPV18. HPV18 is excised from pBluescript II SK+:HPV18 by limited Eco RI digestion and subsequent purification of the 1.3 Kb fragment from agarose gels. The resulting Eco RI HPV18 fragment is ligated into Eco RI-cut, CIP-treated pSZ9016-1. Subclones are selected in which the sense orientation of HPV18 is downstream of the HIV LTR promoter.

d) pAcUW51:HPV18L1 The full-length HPV18L1 ORF was amplified by PCR from clone #187- 1 using oligonucleotide primers providing flanking BglII sites. The L1 gene was inserted into the BamHI site of the baculovirus transfer vector, pAcUW51 (PharMingen, Inc.), under control of the polyhedrin promoter. Recombinant baculoviruses were generated containing the HPV18 L1 expression cassette according to the procedures described in the Pharmingen Manual. Recombinant clones were purified by limiting dilution and dot blot hybridization.

EXAMPLE 18

Expression Of The HPV18 Polypeptide By In Vitro Transcription/ Translation And By Transfection Into Host Cells

Vectors containing HPV DNA sequences are used to drive the translation of the HPV18 polypeptide in rabbit reticulocyte lysates, mammalian host cells, and in baculovirus infected insect cells. The experimental procedures are essentially those outlined in the manufacturers' instructions.

a) In vitro Transcription/Translation. pBluescript III SK+:HPV18 plasmid DNA (with HPV18 in the T7 orientation) is linearized by Bam HI digestion downstream of the HPV18 insert. The linearized plasmid is purified and used as a template for run-off transcription using T7 RNA polymerase in the presence of m7G(5')ppp(5')G. The resulting capped HPV18 transcripts are purified by LiCl precipitation and used to drive the translation of HPV18 in nuclease-pretreated rabbit reticulocyte lysate in the presence of L- [^{35}S] methionine.

b) Expression in Mammalian Cells. The HPV18 protein is expressed in mammalian host cells following transfection with either pcDNA I/Amp:HPV18 (under control of the CMV promoter) or pSZ9016-1:HPV18 (under control of the HIV LTR promoter). In the latter case (pSZ9016-1:HPV18), cells are co-transfected with the TAT expressing plasmid pSZ9016-1:TAT. For both HPV18 expression plasmids, COS-7 cells are transfected using either DEAE-dextran or lipofection with Lipofectamine (BRL).

c) Expression in Insect Cells. The HPV18 L1-containing baculovirus transfer vector pAcUW51:HPV18 L1 is used to produce recombinant baculovirus (Autographa californica) by in vivo homologous recombination. Epitope tagged HPV18 L1 is then expressed in Sf9 (*Spodoptera frugiperda*) insect cells grown in suspension culture following infection with the HPV18-containing recombinant baculovirus.

EXAMPLE 19

Compounds that affect HPV18 activity may be detected by a variety of methods. A method of identifying compounds that affect HPV18 comprises:

(a) mixing a test compound with a solution containing HPV18 to form a mixture;

(b) measuring HPV18 activity in the mixture; and

(c) comparing the HPV18 in the mixture to a standard.

Compounds that affect HPV18 activity may be formulated into pharmaceutical compositions. Such pharmaceutical compositions may be useful for treating diseases or conditions that are characterized by HPV18 infection.

EXAMPLE 20

DNA which is structurally related to DNA encoding HPV18 is detected with a probe. A suitable probe may be derived from DNA having all or a portion of the nucleotide sequence of FIG. 1 or FIG. 3, RNA encoded by DNA having all or a portion of the nucleotide sequence of FIG. 1 or FIG. 3 or degenerate oligonucleotides derived from a portion of the sequence of FIG. 1 or FIG. 3.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 16

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1524 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATGGCTTTGT GGCGGCCTAG TGACAATACC GTATACCTTC CACCTCCTTC TGTGGCAAGA     60
GTTGTAAATA CTGATGATTA TGTGACTCGC ACAAGCATAT TTTATCATGC TGGCAGCTCT    120
AGATTATTAA CTGTTGGTAA TCCATATTTT AGGGTTCCTG CAGGTGGTGG CAATAAGCAG    180
GATATTCCTA AGGTTTCTGC ATACCAATAT AGAGTATTTC GGGTGCAGTT ACCTGACCCA    240
AATAAATTTG GTTTACCTGA TAATAGTATT TATAATCCTG AAACACAACG TTTAGTGTGG    300
GCCTGTGCTG GAGTGGAAAT TGGCCGTGGT CAGCCTTTAG GTGTTGGCCT TAGTGGGCAT    360
CCATTTTATA ATAAATTAGA TGACACTGAA AGTTCCCATG CCGCTACGTC TAATGTTTCT    420
GAGGACGTTA GGGACAATGT GTCTGTAGAT TATAAGCAGA CACAGTTATG TATTTTGGGC    480
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TGTGCCCCTG CTATTGGGGA ACACTGGGCT AAAGGCACTG CTTGTAAATC GCGTCCTTTA   540
TCACAGGGCG ATTGCCCCCC TTTAGAACTT AAGAACACAG TTTTGGAAGA TGGTGATATG   600
GTAGATACTG GATATGGTGC CATGGACTTT AGTACATTGC AAGATACTAA ATGTGAGGTA   660
CCATTGGATA TTTGTCAGTC TATTTGTAAA TATCCTGATT ATTTACAAAT GTCTGCAGAT   720
CCTTATGGGG ATTCCATGTT TTTTTGCTTA CGACGTGAGC AGCTTTTTGC TAGGCATTTT   780
TGGAATAGGG CAGGTACTAT GGGTGACACT GTGCCTCAAT CCTTATATAT TAAAGGCACA   840
GGTATGCGTG CTTCACCTGG CAGCTGTGTG TATTCTCCCT CTCCAAGTGG CTCTATTGTT   900
ACCTCTGACT CCCAGTTGTT TAATAAACCA TATTGGTTAC ATAAGGCACA GGGTCATAAC   960
AATGGTATCT GCTGGCATAA TCAATTATTT GTTACTGTGG TAGATACCAC TCGTAGTACC  1020
AATTTAACAA TATGTGCTTC TACACAGTCT CCTGTACCTG GGCAATATGA TGCTACCAAA  1080
TTTAAGCAGT ATAGCAGACA TGTTGAAGAA TATGATTTGC AGTTTATTTT TCAGTTATGT  1140
ACTATTACTT TAACTGCAGA TGTTATGTCC TATATTCATA GTATGAATAG CAGTATTTTA  1200
GAGGATTGGA ACTTTGGTGT TCCCCCCCCG CCAACTACTA GTTTGGTGGA TACATATCGT  1260
TTTGTACAAT CTGTTGCTAT TACCTGTCAA AAGGATGCTG CACCAGCTGA AAATAAGGAT  1320
CCCTATGATA AGTTAAAGTT TTGGAATGTG GATTTAAAGG AAAAGTTTTC TTTGGACTTA  1380
GATCAATATC CCCTTGGACG TAAATTTTTG GTTCAGGCTG GATTGCGTCG CAAGCCCACC  1440
ATAGGCCCTC GTAAACGTTC TGCTCCATCT GCCACTACGT CTTCTAAACC TGCCAAGCGT  1500
GTGCGTGTAC GTGCCAGGAA GTAA                                         1524
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 507 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Leu Trp Arg Pro Ser Asp Asn Thr Val Tyr Leu Pro Pro Pro
 1               5                  10                  15

Ser Val Ala Arg Val Val Asn Thr Asp Asp Tyr Val Thr Arg Thr Ser
            20                  25                  30

Ile Phe Tyr His Ala Gly Ser Ser Arg Leu Leu Thr Val Gly Asn Pro
        35                  40                  45

Tyr Phe Arg Val Pro Ala Gly Gly Gly Asn Lys Gln Asp Ile Pro Lys
    50                  55                  60

Val Ser Ala Tyr Gln Tyr Arg Val Phe Arg Val Gln Leu Pro Asp Pro
65                  70                  75                  80

Asn Lys Phe Gly Leu Pro Asp Asn Ser Ile Tyr Asn Pro Glu Thr Gln
                85                  90                  95

Arg Leu Val Trp Ala Cys Ala Gly Val Glu Ile Gly Arg Gly Gln Pro
            100                 105                 110

Leu Gly Val Gly Leu Ser Gly His Pro Phe Tyr Asn Lys Leu Asp Asp
        115                 120                 125
```

-continued

```
Thr Glu Ser Ser His Ala Ala Thr Ser Asn Val Ser Glu Asp Val Arg
    130                 135                 140

Asp Asn Val Ser Val Asp Tyr Lys Gln Thr Gln Leu Cys Ile Leu Gly
145                 150                 155                 160

Cys Ala Pro Ala Ile Gly Glu His Trp Ala Lys Gly Thr Ala Cys Lys
                165                 170                 175

Ser Arg Pro Leu Ser Gln Gly Asp Cys Pro Pro Leu Glu Leu Lys Asn
            180                 185                 190

Thr Val Leu Glu Asp Gly Asp Met Val Asp Thr Gly Tyr Gly Ala Met
        195                 200                 205

Asp Phe Ser Thr Leu Gln Asp Thr Lys Cys Glu Val Pro Leu Asp Ile
    210                 215                 220

Cys Gln Ser Ile Cys Lys Tyr Pro Asp Tyr Leu Gln Met Ser Ala Asp
225                 230                 235                 240

Pro Tyr Gly Asp Ser Met Phe Phe Cys Leu Arg Arg Glu Gln Leu Phe
                245                 250                 255

Ala Arg His Phe Trp Asn Arg Ala Gly Thr Met Gly Asp Thr Val Pro
            260                 265                 270

Gln Ser Leu Tyr Ile Lys Gly Thr Gly Met Arg Ala Ser Pro Gly Ser
        275                 280                 285

Cys Val Tyr Ser Pro Ser Pro Ser Gly Ser Ile Val Thr Ser Asp Ser
    290                 295                 300

Glu Leu Phe Asn Lys Pro Tyr Trp Leu His Lys Ala Gln Gly His Asn
305                 310                 315                 320

Asn Gly Ile Cys Trp His Asn Gln Leu Phe Val Thr Val Val Asp Thr
                325                 330                 335

Thr Arg Ser Thr Asn Leu Thr Ile Cys Ala Ser Thr Gln Ser Pro Val
            340                 345                 350

Pro Gly Gln Tyr Asp Ala Thr Lys Phe Lys Gln Tyr Ser Arg His Val
        355                 360                 365

Glu Glu Tyr Asp Leu Gln Phe Ile Phe Gln Leu Cys Thr Ile Thr Leu
    370                 375                 380

Thr Ala Asp Val Met Ser Tyr Ile His Ser Met Asn Ser Ser Ile Leu
385                 390                 395                 400

Glu Asp Trp Asn Phe Gly Val Pro Pro Pro Pro Thr Thr Ser Leu Val
                405                 410                 415

Asp Thr Tyr Arg Phe Val Gln Ser Val Ala Ile Thr Cys Gln Lys Asp
            420                 425                 430

Ala Ala Pro Ala Glu Asn Lys Asp Pro Tyr Asp Lys Leu Lys Phe Trp
        435                 440                 445

Asn Val Asp Leu Lys Glu Lys Phe Ser Leu Asp Leu Asp Gln Tyr Pro
    450                 455                 460

Leu Gly Arg Lys Phe Leu Val Gln Ala Gly Leu Arg Arg Lys Pro Thr
465                 470                 475                 480

Ile Gly Pro Arg Lys Arg Ser Ala Pro Ser Ala Thr Thr Ser Ser Lys
                485                 490                 495

Pro Ala Lys Arg Val Arg Val Arg Ala Arg Lys
            500                 505
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1389 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

-continued

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATGGTATCCC ACCGTGCCGC ACGACGCAAA CGGGCTTCGG TGACTGACTT ATATAAAACA     60
TGTAAACAAT CTGGTACATG TCCATCTGAT GTTGTTAATA AGGTAGAGGG CACCACGTTA    120
GCAGATAAAA TATTGCAATG GTCAAGCCTT GGTATATTTT TGGGTGGACT TGGCATAGGT    180
ACTGGAAGTG GTACAGGGGG TCGTACAGGG TACATTCCAT TGGGTGGGCG TTCCAATACA    240
GTTGTGGATG TCGGTCCTAC ACGTCCTCCA GTGGTTATTG AACCTGTGGG CCCCACAGAC    300
CCATCTATTG TTACATTAAT AGAGGACTCA AGTGTTGTTA CATCAGGTGC ACCTAGGCCT    360
ACTTTTACTG GCACGTCTGG GTTTGATATA ACATCTGCTG GTACAACTAC ACCTGCAGTT    420
TTGGATATCA CACCTTCGTC TACCTCTGTT TCTATTTCCA CAACCAATTT TACCAATCCT    480
GCATTTTCTG ATCCGTCCAT TATTGAAGTT CCACAAACTG GGGAGGTGTC AGGTAATGTA    540
TTTGTTGGTA CCCCTACATC TGGAACACAT GGGTATGAAG AAATACCTTT ACAAACATTT    600
GCTTCTTCTG GTACGGGGGA GGAACCCATT AGTAGTACCC CATTGCCTAC TGTGCGGCGT    660
GTAGCAGGTC CCCGCCTTTA CAGTAGGGCC TACCAACAAG TGTCTGTGGC TAACCCTGAG    720
TTTCTTACAC GTCCATCCTC TTTAATTACC TATGACAACC CGGCCTTTGA GCCTGTGGAC    780
ACTACATTAA CATTTGAGCC TCGTAGTAAT GTTCCTGATT CAGATTTTAT GGATATTATC    840
CGTTTACATA GGCCTGCTTT AACATCCAGG CGTGGTACTG TGCGCTTTAG TAGATTAGGT    900
CAAAGGGCAA CTATGTTTAC CCGTAGCGGT ACACAAATAG GTGCTAGGGT TCACTTTTAT    960
CATGATATAA GTCCTATTGC ACCCTCCCCA GAATATATTG AACTGCAGCC TTTAGTATCT   1020
GCCACGGAGG ACAATGGCTT GTTTGATATA TATGCAGATG ACATAGACCC TGCAATGCCT   1080
GTACCATCGC GTCCTACTAC CTCCTCTGCA GTTTCTACAT ATTCGCCCAC TATATCATCT   1140
GCCTCTTCCT ATAGTAATGT AACGGTCCCT TTAACCTCCT CTTGGGATGT GCCTGTATAC   1200
ACGGGTCCTG ATATTACATT ACCACCTACT ACCTCTGTAT GGCCCATTGT ATCACCCACA   1260
GCCCCTGCCT CTACACAGTA TATTGGTATA CATGGTACAC ATTATTATTT GTGGCCATTA   1320
TATTATTTTA TTCCTAAAAA GCGTAAACGT GTTCCCTATT TTTTTGCAGA TGGCTTTGTG   1380
GCGGCCTAG                                                           1389
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 461 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(v i) ORIGINAL SOURCE:

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Ser His Arg Ala Ala Arg Arg Lys Arg Ala Ser Val Thr Asp
1 5 10 15

Leu Tyr Lys Thr Cys Lys Gln Ser Gly Thr Cys Pro Ser Asp Val Val
20 25 30

Asn Lys Val Glu Gly Thr Thr Leu Ala Asp Lys Ile Leu Gln Trp Ser
35 40 45

Ser Leu Gly Ile Phe Leu Gly Gly Leu Gly Ile Gly Thr Gly Ser Gly
50 55 60

Thr Gly Gly Arg Thr Gly Tyr Ile Pro Leu Gly Gly Arg Ser Asn Thr
65 70 75 80

Val Val Asp Val Gly Pro Thr Arg Pro Pro Val Val Ile Glu Pro Val
85 90 95

Gly Pro Thr Asp Pro Ser Ile Val Thr Leu Ile Glu Asp Ser Ser Val
100 105 110

Val Thr Ser Gly Ala Pro Arg Pro Thr Phe Thr Gly Thr Ser Gly Phe
115 120 125

Asp Ile Thr Ser Ala Gly Thr Thr Thr Pro Ala Val Leu Asp Ile Thr
130 135 140

Pro Ser Ser Thr Ser Val Ser Ile Ser Thr Thr Asn Phe Thr Asn Pro
145 150 155 160

Ala Phe Ser Asp Pro Ser Ile Ile Glu Val Pro Gln Thr Gly Glu Val
165 170 175

Ser Gly Asn Val Phe Val Gly Thr Pro Thr Ser Gly Thr His Gly Tyr
180 185 190

Glu Glu Ile Pro Leu Gln Thr Phe Ala Ser Ser Gly Thr Gly Glu Glu
195 200 205

Pro Ile Ser Ser Thr Pro Leu Pro Thr Val Arg Arg Val Ala Gly Pro
210 215 220

Arg Leu Tyr Ser Arg Ala Tyr Gln Gln Val Ser Val Ala Asn Pro Glu
225 230 235 240

Phe Leu Thr Arg Pro Ser Ser Leu Ile Thr Tyr Asp Asn Pro Ala Phe
245 250 255

Glu Pro Val Asp Thr Thr Leu Thr Phe Glu Pro Arg Ser Asn Val Pro
260 265 270

Asp Ser Asp Phe Met Asp Ile Ile Arg Leu His Arg Pro Ala Leu Thr
275 280 285

Ser Arg Arg Gly Thr Val Arg Phe Ser Arg Leu Gly Gln Arg Ala Thr
290 295 300

Met Phe Thr Arg Ser Gly Thr Gln Ile Gly Ala Arg Val His Phe Tyr
305 310 315 320

His Asp Ile Ser Pro Ile Ala Pro Ser Pro Glu Tyr Ile Glu Leu Gln
325 330 335

Pro Leu Val Ser Ala Thr Glu Asp Asn Gly Leu Phe Asp Ile Tyr Ala
340 345 350

Asp Asp Ile Asp Pro Ala Met Pro Val Pro Ser Arg Pro Thr Thr Ser
355 360 365

Ser Ala Val Ser Thr Tyr Ser Pro Thr Ile Ser Ser Ala Ser Ser Tyr
370 375 380

Ser Asn Val Thr Val Pro Leu Thr Ser Ser Trp Asp Val Pro Val Tyr
385 390 395 400

Thr Gly Pro Asp Ile Thr Leu Pro Pro Thr Ser Val Trp Pro Ile Val
405 410 415

-continued

```
Ser Pro Thr Ala Pro Ala Ser Thr Gln Tyr Ile Gly Ile His Gly Thr
            420                 425                 430

His Tyr Tyr Leu Trp Pro Leu Tyr Tyr Phe Ile Pro Lys Lys Arg Lys
        435                 440                 445

Arg Val Pro Tyr Phe Phe Ala Asp Gly Phe Val Ala Ala
    450                 455                 460
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAAGATCTCA CAAAACAAAA TGGCTTTGTG GCGGCCTAGT G 41

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAAGATCTTT ACTTCCTGGC ACGTACACGC ACACGC 36

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCCCCGGGC ACAAAACAAA ATGGTATCCC ACCGTGCCGC ACGAC 45

-continued

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TCCCCCGGGC TAGGCCGCCA CAAAGCCATC TGC 33

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAATCCTTAT ATATTAAAGG CACAGGTATG 30

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATCATATTG CCCAGGTACA GGAGACTGTG 30

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

-continued

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAAGATCTCA CAAAACAAAA TGGCTTTGTG GCGGCCTAGT G 41

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCTAACGTCC TCAGAAACAT TAGAC 25

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTTAAAGCTT ATGTCACTTT CTCTTGTATC 30

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TGATAAGCTT GCTCAATGGT TCTCTTCCTC 30

-continued

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TGGTCATCCC AAATCTTGAA A 21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CACCGTAGTG TTTGGAAGCG A 21

What is claimed is:

1. Isolated or purified virus-like particles comprising recombinant Human Papillomavirus type 18 L1 protein having the amino acid sequence of SEQ ID No: 2.

2. The virus-like particles of claim 1 which further comprise recombinant L2 protein.

3. The virus-like particles of claim 1, wherein said particles are produced by expression of a recombinant nucleic acid encoding SEQ ID NO: 2.

4. The virus-like particles of claim 3, wherein said particles are produced by expression of a recombinant nucleic acid encoding SEQ ID NO: 1.

5. A vaccine comprising a pharmaceutically acceptable carrier and an immunoprotective amount of the virus-like particles of claim 1.

6. A vaccine comprising a pharmaceutically acceptable carrier and an immunoprotective amount of the virus-like particles of claim 2.

7. A method of preventing papillomavirus infection comprising administering the vaccine of claim 5 to a host.

8. A method of preventing papillomavirus infection comprising administering the vaccine of claim 6 to a host.

9. A method for producing the virus-like particles of claim 1, comprising:

(a) preparing a vector comprising a DNA molecule of SEQ ID NO:1;

(b) transforming a host cell with the vector of step (a) to produce a transformed cell;

(c) cultivating the transformed cell under conditions that permit production of recombinant human papillomavirus L1 protein; and

(d) purifying the protein under conditions that permit formation of the virus-like proteins.

10. A method of inducing an immune response in an animal comprising administering the virus-like particle claim 1 to the animal.

11. Isdated or purified virus-like particles comprised of recombinant human papillomavirus type 18 L1 protein, the L1 protein having amino acid R at position 30, amino acid N at position 88, amino acid R at position 283 and amino acid R at position 338.

* * * * *

EXHIBIT 5
2 PAGES

EXHIBIT 5

United States Patent and Trademark Office

Maintenance Fee Statement 07/13/2006 09:34 AM EDT

Patent Number: 5820870

Customer Number: 000000

CHRISTINE E CARTY
PATENT DEPT
MERCK AND CO INC
P O BOX 2000
RAHWAY NJ 07065-0907

The data shown below is from the records of the U.S. Patent and Trademark Office. If the maintenance fee and any necessary surcharge have been timely paid for the patent listed below, the notation "PAID" will appear in the "STAT" column.

If the statement of small entity status is defective the reason will be indicated below in the "Small Entity" status column. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

PATENT NUMBER	FEE AMT	SUR-CHARGE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	STAT	ATTY DKT NUMBER
5,820,870	$880.00	$0.00	08/409,122	10/13/98	03/22/95	04	NO	PAID	19425

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Maintenance Fee Statement 07/13/2006 09:34 AM EDT

Patent Number: 5820870

Customer Number: 000000

CHRISTINE E CARTY
PATENT DEPT
MERCK AND CO INC
P O BOX 2000
RAHWAY NJ 07065-0907

The data shown below is from the records of the U.S. Patent and Trademark Office. If the maintenance fee and any necessary surcharge have been timely paid for the patent listed below, the notation "PAID" will appear in the "STAT" column.

If the statement of small entity status is defective the reason will be indicated below in the "Small Entity" status column. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

PATENT NUMBER	FEE AMT	SUR-CHARGE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	STAT	ATTY DKT NUMBER
5,820,870	$2,300.00	$0.00	08/409,122	10/13/98	03/22/95	08	NO	PAID	19425

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Alexandria, VA 22313-1450

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EXHIBIT 6
2 PAGES

```
      1030        1040        1050        1060        1070        1080
AATTTAACAA TATGTGCTTC TACACAGTCT CCTGTACCTG GGCAATATGA TGCTACCAAA
TTAAATTGTT ATACACGAAG ATGTGTCAGA GGACATGGAC CCGTTATACT ACGATGGTTT

      1090        1100        1110        1120        1130        1140
TTTAAGCAGT ATAGCAGACA TGTTGAAGAA TATGATTTGC AGTTTATTTT TCAGTTATGT
AAATTCGTCA TATCGTCTGT ACAACTTCTT ATACTAAACG TCAAATAAAA AGTCAATACA

      1150        1160        1170        1180        1190        1200
ACTATTACTT TAACTGCAGA TGTTATGTCC TATATTCATA GTATGAATAG CAGTATTTTA
TGATAATGAA ATTGACGTCT ACAATACAGG ATATAAGTAT CATACTTATC GTCATAAAAT

      1210        1220        1230        1240        1250        1260
GAGGATTGGA ACTTTGGTGT TCCCCCCCCG CCAACTACTA GTTTGGTGGA TACATATCGT
CTCCTAACCT TGAAACCACA AGGGGGGGGC GGTTGATGAT CAAACCACCT ATGTATAGCA

      1270        1280        1290        1300        1310        1320
TTTGTACAAT CTGTTGCTAT TACCTGTCAA AAGGATGCTG CACCAGCTGA AAATAAGGAT
AAACATGTTA GACAACGATA ATGGACAGTT TTCCTACGAC GTGGTCGACT TTTATTCCTA

      1330        1340        1350        1360        1370        1380
CCCTATGATA AGTTAAAGTT TTGGAATGTG GATTTAAAGG AAAAGTTTTC TTTGGACTTA
GGGATACTAT TCAATTTCAA AACCTTACAC CTAAATTTCC TTTTCAAAAG AAACCTGAAT

      1390        1400        1410        1420        1430        1440
GATCAATATC CCCTTGGACG TAAATTTTTG GTTCAGGCTG GATTGCGTCG CAAGCCCACC
CTAGTTATAG GGGAACCTGC ATTTAAAAAC CAAGTCCGAC CTAACGCAGC GTTCGGGTGG

      1450        1460        1470        1480        1490        1500
ATAGGCCCTC GTAAACGTTC TGCTCCATCT GCCACTACGT CTTCTAAACC TGCCAAGCGT
TATCCGGGAG CATTTGCAAG ACGAGGTAGA CGGTGATGCA GAAGATTTGG ACGGTTCGCA

      1510        1520        1530        1540        1550        1560
GTGCGTGTAC GTGCCAGGAA GTAA..... .......... .......... ..........
CACGCACATG CACGGTCCTT CATT..... .......... .......... ..........
```

Figure 7.9: Deduced Polypeptide Sequence of the HPV18L1 Protein

```
        10          20          30          40          50          60
MALWRPSDNT VYLPPPSVAR VVNTDDYVTR TSIFYHAGSS RLLTVGNPYF RVPAGGGNKQ

        70          80          90         100         110         120
DIPKVSAYQY RVFRVQLPDP NKFGLPDNSI YNPETQRLVW ACAGVEIGRG QPLGVGLSGH

       130         140         150         160         170         180
PFYNKLDDTE SSHAATSNVS EDVRDNVSVD YKQTQLCILG CAPAIGEHWA KGTACKSRPL

       190         200         210         220         230         240
SQGDCPPLEL KNTVLEDGDM VDTGYGAMDF STLQDTKCEV PLDICQSICK YPDYLQMSAD

       250         260         270         280         290         300
PYGDSMFFCL RREQLFARHF WNRAGTMGDT VPQSLYIKGT GMRASPGSCV YSPSPSGSIV
```

```
       310         320         330         340         350         360
TSDSQLFNKP YWLHKAQGHN NGICWHNQLF VTVVDTTRST NLTICASTQS PVPGQYDATK

       370         380         390         400         410         420
FKQYSRHVEE YDLQFIFQLC TITLTADVMS YIHSMNSSIL EDWNFGVPPP PTTSLVDTYR

       430         440         450         460         470         480
FVQSVAITCQ KDAAPAENKD PYDKLKFWNV DLKEKFSLDL DQYPLGRKFL VQAGLRRKPT

       490         500         510         520         530         540
IGPRKRSAPS ATTSSKPAKR VRVRARK*.. .......... .......... ..........
```

*Denotes stop codon.

A.2. Expression Vectors for the Four HPV L1 Proteins

Figure 7.10 illustrates the salient features of the high copy number, autonomously replicating, leucine-selectable shuttle vector pGAL110-HPV6aL1. This vector (13.6 kbp) contains the entire yeast 2 micron DNA with the yeast *LEU2* gene inserted as a selective marker. The 2 micron DNA is joined to a 4.4 kbp *Eco*RI-linearized pBR322 plasmid containing the ampicillin resistance (Amp^r) gene and the origin of replication. The expression cassette is made up of the promoter from the yeast galactokinase gene (*GAL1*p) and transcriptional terminator from the yeast alcohol dehydrogenase I gene (*ADH1*t) flanking the ORF encoding the HPV6L1 polypeptide. Details of construction of this vector are given in Section 7.1.C. and Appendix 1. The expression vector backbone (without HPV ORF) is identical to the above for the other three HPV types. The difference for each vector is the presence of the appropriate HPV L1 ORF as shown in Figures 7.10 to 7.13

EXHIBIT 7
3 PAGES

EXHIBIT 7

DEPARTMENT OF HEALTH & HUMAN SERVICES

DR. C. RUSSO
APR 2 4 2000

Public Health Service

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

APR 19 2000

Our Reference: BB-**IND 9030**

Division of Vaccines and
Related Products Applications
Telephone: (301) 827-3070

Merck and Company, Inc.
Attention: Carlo Russo, M.D.
Sumneytown Pike
P.O. Box 4, UN-B121
West Point, PA 19486

Dear Dr. Russo:

The Center for Biologics Evaluation and Research has received your **Investigational New Drug Application (IND)**. The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND#: **9030**

SPONSOR: **Merck and Company, Inc.**

PRODUCT NAME: **Human Papillomavirus Quadrivalent (Types 6, 11, 16 and 18; *S. cerevisiae*) L1 Capsid Virus Like Particle Vaccine with Alum**

DATE OF SUBMISSION: **April 14, 2000**

DATE OF RECEIPT: **April 14, 2000**

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an **original and two copies of every submission to this file, including the Form FDA 1571**. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above, unless a waiver has been requested and granted. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file you will be notified in writing of the reasons for placing the IND on hold.

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.
Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect. Any unexpected fatal or immediately life-threatening reaction which is associated with use of this product must be reported to this Center within seven calendar days, and all serious, unexpected adverse experiences must be reported, in writing, to this Center and to all study centers within fifteen calendar days.

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of Form FDA 1571 requests that either an environmental assessment, or a claim for categorical exclusion from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one.

Sponsors of INDs for products used to treat life-threatening or severely-debilitating illnesses are encouraged to consider the interim rule outlined in 21 CFR 312.80 through 312.88.

Telephone inquiries concerning this IND should be made directly to this Division at (301) 827-3070. Correspondence regarding this file should be addressed as follows:

Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Vaccines Research and Review
Division of Vaccines and Related Products Applications
1401 Rockville Pike
HFM-99, Suite 200 North
Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Stephanie Milwit

Stephanie Milwit
Regulatory Information Specialist
Division of Vaccines and
Related Products Applications
Office of Vaccines
Research and Review
Center for Biologics
Evaluation and Research

Enclosures (5) - 21 CFR 312
21 CFR 50.20, 50.25
FDA Forms 1571 & 1572

EXHIBIT 8
2 PAGES

EXHIBIT 8

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

Attn: Patrick Brill-Edwards, M.D.
Merck & Co., Inc.
P.O. Box 4, BLB-22
Sumneytown Pike
West Point, PA 19486

December 12, 2005

Dear Dr. Brill-Edwards:

We have received your biologics license application (BLA) submitted under section 351 of the Public Health Service Act for the following biological product:

Our Submission Tracking Number (STN): BL 125126/0

Biological Product: Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine

Indication: Active immunization for the prevention of disease against Human Papillomavirus (Types 6, 11, 16, 18)

Date of Supplement: December 6, 2005

Date of Receipt: December 7, 2005

First Action Due Date: June 8, 2005

US License Number: 0002

Please submit all future correspondence, supporting data, or labeling relating to this application in triplicate, citing the above STN number. Send all correspondence to the following address:

Norman Baylor, Ph.D., HFM-475
Center for Biologics Evaluation and Research
Food and Drug Administration
Suite 200N
1401 Rockville Pike
Rockville, MD 20852-1448

We will notify you within 60 days of the receipt date if the application is sufficiently complete to permit a substantive review.

If you have any questions, please contact Gopa Raychaudhuri, Regulatory Project Manager, at (301) 827-3070.

Sincerely yours,

Loris D. McVittie

Loris D. McVittie, Ph.D.
Chief
Viral Vaccine Branch
Division of Vaccines and
Related Products Applications
Office of Vaccines
Research and Review
Center for Biologics
Evaluation and Research

EXHIBIT 9
7 PAGES

EXHIBIT 9

Restricted Confidential limited access

Date	Event Description	Reg. No.
08-Jun-2006	GARDASIL approval letter	BLA 125126
08-Jun-2006	Post-licensure clinical commitments sent by secure email on June 6, 2006	BLA 125126
08-Jun-2006	Response to question regarding stability studies received on June 7, 2006	BLA 125126
06-Jun-2006	Response to question regarding final container samples received on May 31, 2006	BLA 125126
02-Jun-2006	Product-related post-marketing commitments	BLA 125126
01-Jun-2006	Response to question regarding PCR positive and sero-positive patients received on May 19, 2006	BLA 125126
31-May-2006	Response to questions regarding raw materials used in manufacture received on May 24, 2006	BLA 125126
30-May-2006	Clinical post-licensure commitments	BLA 125126
28-May-2006	Product-related post-marketing commitments	BLA 125126
26-May-2006	Responses to questions regarding stability data received on May 23, 2006	BLA 125126
25-May-2006	Merck presentation for May 18, 2006 VRBPAC meeting	BLA 125126
23-May-2006	Responses to questions regarding the pharmacovigilance plan received on May 4, 2006	BLA 125126
22-May-2006	IND Annual report	BBIND 9030
18-May-2006	VRBPAC Background Document GARDASIL	BLA 125126
17-May-2006	Responses to questions regarding Combined Analysis and CIN 2/3 received on May 1, 2006.	BLA 125126
11-May-2006	Safety Report - Initial Written Report	BBIND 9030
09-May-2006	Responses to questions regarding the Safety Update Report received on April 27, 2006	BLA 125126
08-May-2006	Resubmission of Response to Inspection questions received on April 19, 2006	BLA 125126
08-May-2006	CBER'S comments on the proposed Pharmacovigilance plan	BLA 125126
04-May-2006	Responses to Clinical assay questions received on March 30, 2006	BLA 125126
03-May-2006	Response to CMC questions regarding Table 3.2.S.2.5.4 received on April 27, 2006	BLA 125126
03-May-2006	Submission of line numbered version of the Draft Labeling Text	BLA 125126
02-May-2006	Responses to clinical questions regarding protocol 005 received on April 21, 2006	BLA 125126
02-May-2006	Response to Inspection questions received on April 19, 2006.	BLA 125126
27-Apr-2006	FDA Statistical Review and Evaluation for ACM	BLA 125126
27-Apr-2006	Response to clinical question regarding protocol 013 received on April 24, 2006	BLA 125126
26-Apr-2006	Responses to Pre- Clinical and Statistical questions received on March 28, 2006	BLA 125126
24-Apr-2006	Responses to clinical questions received on March 28, 2006	BLA 125126
21-Apr-2006	Final ACM briefing document	BLA 125126
17-Apr-2006	Overview of Safety Update Report as requested by CBER on April 4, 2006	BLA 125126
17-Apr-2006	Safety Report - Follow-up to a Written Report	BBIND 9030
13-Apr-2006	Safety Report - Follow-up to a Written Report	BBIND 9030
13-Apr-2006	Response to clinical question received on April 5, 2006	BLA 125126
13-Apr-2006	Responses to CMC questions received on March 28, 2006	BLA 125126
07-Apr-2006	Responses to clinical questions received on March 15, 2006	BLA 125126
06-Apr-2006	Draft Background Document for GARDASIL ACM	BLA 125126

03-Apr-2006	Safety Update Report for GARDASIL	BLA 125126
30-Mar-2006	Responses to clinical questions received on March 1, 2006	BLA 125126
28-Mar-2006	Safety Report - Follow-up to a Written Report	BBIND 9030
28-Mar-2006	CBER'S comments regarding draft release protocols	BLA 125126
27-Mar-2006	Safety Reports - Initial Written Reports	BBIND 9030
24-Mar-2006	Safety Report - Initial Written Report	BBIND 9030
23-Mar-2006	Follow-up commitment to update 4 sections of the license application	BLA 125126
22-Mar-2006	Responses to Analysis Oriented questions sent by CBER by secure email on March 1, 2006.	BLA 125126
17-Mar-2006	Safety Reports - Initial Written Reports	BBIND 9030
13-Mar-2006	Complete Protocol Amendment 020-03	BBIND 9030
11-Mar-2006	Email from CBER concerning Clinical questions	BLA 125126
06-Mar-2006	Clinical questions from CBER regarding the BLA sent by secure email on March 1, 2006	BLA 125126
06-Mar-2006	Response to request for CMC information	BLA 125126
06-Mar-2006	Responses to pre-approval inspection	BLA 125126
03-Mar-2006	Safety Reports - Follow-up to Written Reports	BBIND 9030
01-Mar-2006	IND Annual Report	BBIND 8841
24-Feb-2006	Safety Report - Follow-up to a Written Report	BBIND 9030
15-Feb-2006	Safety Report - Follow-up to a Written Report	BBIND 9030
15-Feb-2006	Safety Report - Initial Written Report	BBIND 9030
15-Feb-2006	CBER questions on CMC	BLA 125126
10-Feb-2006	Safety Report - Follow-up to a Written Report	BBIND 9030
09-Feb-2006	Safety Report - Follow-up to a Written Report	BBIND 9030
08-Feb-2006	Safety Report - Follow-up to a Written Report	BBIND 9030
08-Feb-2006	Amendment to Pending Application-- Control Procedures and an SOP for drug substance and drug product	BLA 125126
03-Feb-2006	Safety Report - Initial Written Report	BBIND 9030
02-Feb-2006	Acknowledgment letter of receipt of original BLA	BLA 125126
02-Feb-2006	New Protocol-- Protocol 025	BBIND 9030
02-Feb-2006	Safety Report - Initial Written Report	BBIND 9030
26-Jan-2006	Response to question concerning adverse experience in Protocol 015	BLA 125126
13-Jan-2006	Amendment to Pending Application-- Resubmit CSR 013, § 7.1.2.4 omitted	BLA 125126
09-Jan-2006	Response to FDA Request for Information-- Raw data in SAS transport format for the release assays.	BBIND 9030
27-Dec-2005	Response to FDA Request for Information-- Responses to questions from CBER regarding Protocol 025	BBIND 9030
27-Dec-2005	Additional information on Protocol 007 as requested by CBER	BLA 125126
20-Dec-2005	CBER request for additional CMC information.	BLA 125126
20-Dec-2005	Annual Report for BBIND 7395	BBIND 7395
12-Dec-2005	FDA Acknowledgment letter, GARDASIL review date from CBER	BLA 125126
09-Dec-2005	Submission of Information regarding additional detail to the validation summaries for all equipment	BLA 125126
06-Dec-2005	Revised Cover Letter for the GARDASIL BLA	BLA 125126
02-Dec-2005	Safety Report - Initial Written Report	BBIND 9030
01-Dec-2005	Safety Report - Follow-up to a Written Report	BBIND 9030
01-Dec-2005	Original BLA for Quadrivalent HPV	BLA 125126

30-Nov-2005	Complete Protocol Amendment 019-02	BBIND 9030
29-Nov-2005	Safety Report - Follow-up to a Written Report	BBIND 9030
28-Nov-2005	Complete Protocol Amendment 018-05	BBIND 9030
21-Nov-2005	Safety Report - Follow-up to a Written Report	BBIND 9030
21-Nov-2005	Safety Report - Initial Written Report	BBIND 7218
21-Nov-2005	Safety Report - Initial Written Report	BBIND 7395
21-Nov-2005	Safety Report - Initial Written Report	BBIND 8841
14-Nov-2005	Questions from CBER concerning Protocols 019 and 020.	BBIND 9030
09-Nov-2005	Safety Reports - Initial Written Reports	BBIND 9030
08-Nov-2005	General Correspondence --Response to CBERs summary of the 6/10/05 Pre-BLA Meeting	BBIND 9030
08-Nov-2005	Amendment to Pending Application-- Information regarding Reproductive Toxicity Testing.	BLA 125126
07-Oct-2005	Protocol Amendment 013-05	BBIND 9030
21-Sep-2005	Protocol Amendment 015-05. Addition of month 48 serum collection time point	BBIND 9030
09-Sep-2005	Resubmission of CSR Protocol 005 due to publishing errors.	BLA 125126
31-Aug-2005	Original Application--Submission - CMC and Non-Clinical Sections	BLA 125126
16-Aug-2005	General Correspondence --Seeking CBER concurrence with the design and endpoints of Protocol 025	BBIND 9030
12-Aug-2005	General Correspondence-- Plans for un-blinding and analysis of the Phase III program	BBIND 9030
03-Aug-2005	Annual Report for BBIND7218	BBIND 7218
01-Aug-2005	Original Application --Fast Track submission of the early Clinical CSRs (001, 002, 004, 005, 006, 007)	BLA 125126
28-Jul-2005	General Correspondence --Revised timetable of target dates for each section to be submitted as part of a rolling BLA	BBIND 9030
27-Jul-2005	Safety Reports - Follow-up to a Written Reports	BBIND 9030
21-Jul-2005	Response to FDA Request for Information --Response to FDA regarding product characterization	BBIND 9030
19-Jul-2005	Safety Reports - Follow-up to Written Reports	BBIND 9030
18-Jul-2005	Protocol Amendment --Protocol 020-02, R dated 7/5/05 to correct the exclusion criteria.	BBIND 9030
18-Jul-2005	General Correspondence -Timetable of target dates for each section to be submitted as part of a rolling BLA.	BBIND 9030
15-Jul-2005	Safety Reports - Initial Written Reports	BBIND 9030
14-Jul-2005	Safety Report – Follow-up to a Written Report	BBIND 9030
11-Jul-2005	Safety Report – Follow-up to a Written Report	BBIND 9030
08-Jul-2005	CBERs Pre-BLA Meeting Summary	BBIND 9030
05-Jul-2005	Safety Report - Follow-up to a Written Report	BBIND 9030
14-Jun-2005	Protocol Amendment 020-02.	BBIND 9030
13-Jun-2005	Safety Reports - Follow-up to Written Reports	BBIND 9030
09-Jun-2005	Safety Reports – Follow-up to Written Reports	BBIND 9030
27-May-2005	Safety Report - Initial Written Report	BBIND 9030
27-May-2005	Safety Reports - Follow-up to a Written Reports	BBIND 9030
23-May-2005	Annual report for BBIND9030	BBIND 9030
23-May-2005	Safety Reports – Follow-up to Written Reports	BBIND 9030
23-May-2005	Questions from CBER sent on May 23, 2005 regarding product	BBIND 9030

	characterization.	
29-Apr-2005	CBER notice of HPV face-to-face Pre-BLA meeting date and time.	BBIND 9030
28-Apr-2005	Response to FDA Request for Information --Responses to FDA regarding Mid-Adult Women's Study, Protocol 019	BBIND 9030
26-Apr-2005	Response to FDA Request for Information --Responses to questions regarding the Men's Study, Protocol 020	BBIND 9030
21-Apr-2005	Revised Pre-BLA Background Document	BBIND 9030
15-Apr-2005	Safety Report - Initial Written Report	BBIND 9030
15-Apr-2005	Initial 3500\CIOMS\VAERS form	BBIND 9030
13-Apr-2005	Safety Reports – Follow-up to Written Reports	BBIND 9030
11-Apr-2005	Safety Reports – Initial Written Reports	BBIND 9030
31-Mar-2005	Complete Protocol Amendment 019-01	BBIND 9030
29-Mar-2005	Complete Protocol Amendment 013-04	BBIND 9030
22-Mar-2005	Pre-BLA Background Document	BBIND 9030
09-Mar-2005	Complete Protocol Amendment 015-04	BBIND 9030
03-Mar-2005	Response to FDA Request for Information --Additional Information regarding Reproductive Toxicity testing	BBIND 9030
16-Feb-2005	General Correspondence --Validation Reports of the PCR and cRIA assays	BBIND 9030
10-Feb-2005	IND Annual Report	BBIND 8841
21-Jan-2005	Safety Reports - Follow-up to Written Reports	BBIND 9030
20-Jan-2005	Safety Report - Initial Written Report	BBIND 9030
20-Jan-2005	Initial 3500\CIOMS\VAERS form	BBIND 9030
18-Jan-2005	Safety Report – Follow-up to a Written Report	BBIND 9030
17-Jan-2005	Safety Report - Follow-up to a Written Report	BBIND 9030
10-Jan-2005	Questions from CBER sent on 29 December, 2004 regarding the Mid-Adult Women's Study	BBIND 9030
31-Dec-2004	Questions from CBER sent on 31 Dec., 2004 regarding Protocol 020	BBIND 9030
23-Dec-2004	Safety Report - Initial Written Report.	BBIND 9030
17-Dec-2004	FDA Conversation Record	BBIND 9030
17-Dec-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
15-Dec-2004	Response to FDA Request for Information -CBERs request for additional information 15 November, 2004	BBIND 9030
14-Dec-2004	Protocol Amendment- Protocol 007-10, R dated 23 Nov., 2004	BBIND 9030
08-Dec-2004	IND Annual Report for BBIND 7395	BBIND 7395
08-Dec-2004	Safety Reports - Initial Written Reports	BBIND 9030
03-Dec-2004	General Correspondence- Adverse Experience, Protocol 011.	BBIND 9030
15-Nov-2004	FDA Request for Information. Phase III Immunogenicity Hypothesis.	BBIND 9030
01-Nov-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
22-Oct-2004	Response to FDA Request for Information -proposals and relevant data to support Phase III immunogenicity hypotheses	BBIND 9030
11-Oct-2004	Response to FDA Request for Information -Information on Analytical Methods	BBIND 9030
07-Oct-2004	Protocol Amendment-- Protocol 007-10, R dated 24-Sep-04	BBIND 9030
05-Oct-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
28-Sep-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
24-Sep-2004	Certificate of Confidentiality for Protocol 020	BBIND 9030
21-Sep-2004	General Correspondence -- Request for Certificate of Confidentiality for	BBIND 9030

	Protocol 020.	
16-Sep-2004	Safety Reports – Follow-up to Written Reports	BBIND 9030
15-Sep-2004	Complete Protocol Amendment 018-03	BBIND 9030
15-Sep-2004	General Correspondence- Submission of Rationale regarding GMT issues with the Luminex Assay	BBIND 9030
10-Sep-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
02-Sep-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
23-Aug-2004	General Correspondence-- Clinical Development Plan for the male program	BBIND 9030
18-Aug-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
09-Aug-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
09-Aug-2004	IND Annual Report	BBIND 7218
05-Aug-2004	Protocol 020-HPV Vaccine Efficacy Study In Young Men	BBIND 9030
29-Jul-2004	Response to CBERs request sent 19 May 04.	BBIND 9030
01-Jul-2004	Protocol amendments 011-03, 012-03, and 013-03.	BBIND 9030
22-Jun-2004	FDA Correspondence- CBER's comments on Protocol 019.	BBIND 9030
21-Jun-2004	Protocol Amendment-- Protocol 019, R dated, removal of ECC	BBIND 9030
19-Jun-2004	FDA Questions regarding the Luminex assay report.	BBIND 9030
17-Jun-2004	Annual Report-- BBIND 9030	BBIND 9030
11-Jun-2004	Protocol Amendment --Protocol 016-02, revision of assay to Luminex	BBIND 9030
09-Jun-2004	Protocol Amendment-- Protocol 015-03, revision of assay to Luminex	BBIND 9030
14-May-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
13-May-2004	General Correspondence-- References for background document regarding characterization of the HPV vaccine.	BBIND 9030
05-May-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
30-Apr-2004	Protocol Amendment --Protocol 019-Mid Adult Women's Efficacy Study	BBIND 9030
09-Apr-2004	Safety Reports – Follow-up to Written Reports	BBIND 9030
06-Apr-2004	Safety Report - Follow-up to a Written Report	BBIND 9030
01-Apr-2004	Safety Report - Initial Written Report	BBIND 9030
31-Mar-2004	Safety Report - Initial Written Report	BBIND 9030
23-Mar-2004	Complete Protocol Amendment, 012-02 and 013-02. The removal of neutralization testing.	BBIND 9030
22-Mar-2004	Safety Report – Follow-up to a Written Report	BBIND 9030
22-Mar-2004	Complete Protocol Amendment, 015-02	BBIND 9030
19-Mar-2004	Safety Report - Follow-up to a Written Report	BBIND 9030
19-Mar-2004	Response to FDA Request for Information -Validation Report for the Luminex assay.	BBIND 9030
08-Mar-2004	Safety Report - Follow-up to a Written Report	BBIND 9030
04-Mar-2004	Response to FDA Request for Information -Responses regarding Protocol 016, amendment 01	BBIND 9030
01-Mar-2004	IND Annual Report	BBIND 8841
13-Feb-2004	Safety Report - Follow-up to a Written Report	BBIND 9030
10-Feb-2004	Safety Report - Follow-up to a Written Report	BBIND 9030
06-Feb-2004	Protocol Extension, 007-010	BBIND 9030
05-Feb-2004	Complete Protocol Amendment, 007-06.	BBIND 9030
05-Feb-2004	Safety Report - Follow-up to a Written Report	BBIND 9030
29-Jan-2004	Safety Report - Follow-up to a Written Report	BBIND 9030

21-Jan-2004	Safety Report - Follow-up to a Written Report	BBIND 9030
12-Jan-2004	Safety Report - Initial Written Report	BBIND 9030
23-Dec-2003	IND Annual Report for BBIND 7395.	BBIND 7395
15-Dec-2003	General Correspondence- Background document for the Characterization of the HPV vaccine.	BBIND 9030
25-Nov-2003	Response to FDA Request for Information-6/4/03.	BBIND 9030
25-Nov-2003	Safety Report - Follow-up to a Written Report	BBIND 9030
12-Nov-2003	Safety Report - Follow-up to a Written Report	BBIND 9030
03-Nov-2003	Safety Reports - Follow-up to a Written Reports	BBIND 9030
22-Oct-2003	Safety Report - Follow-up to a Written Report	BBIND 9030
13-Oct-2003	Complete Protocol Amendment, 018, R dated 02 Oct 2003	BBIND 9030
09-Oct-2003	Safety Report - Initial Written Report	BBIND 9030
24-Sep-2003	Complete Protocol Amendment, 016-01, R dated 15-Sep 2003	BBIND 9030
18-Sep-2003	General Correspondence- Intramuscular Developmental Toxicity and Immunogenicity Study in Rats	BBIND 9030
18-Sep-2003	Safety Report - Follow-up to a Written Report	BBIND 9030
15-Sep-2003	Safety Report - Initial Written Report	BBIND 9030
15-Sep-2003	Safety Report - Follow-up to a Written Report	BBIND 9030
11-Sep-2003	Complete Protocol Amendment, 016-01	BBIND 9030
10-Sep-2003	Protocol Amendment	BBIND 9030
04-Sep-2003	Safety Report - Follow-up to a Written Report	BBIND 9030
04-Sep-2003	Safety Report - Initial Written Report	BBIND 9030
25-Aug-2003	Safety Report-- Follow-up to a Written Report	BBIND 9030
14-Aug-2003	IND Annual Report	BBIND 7218
12-Aug-2003	Safety Reports- Initial Written Reports	BBIND 9030
30-Jul-2003	Protocol Amendment - New Protocol 018-00, Adolescent Safety Study	BBIND 9030
24-Jul-2003	Safety Report Follow-up Safety Update	BBIND 9030
11-Jul-2003	Safety Report Follow-up Safety Update	BBIND 9030
27-Jun-2003	Safety Report Follow-up Safety Update	BBIND 9030
27-Jun-2003	Safety Report- Initial Safety Update	BBIND 9030
20-Jun-2003	Safety Report - Follow-up to a Written Report	BBIND 9030
17-Jun-2003	Safety Report- Initial Safety Update	BBIND 9030
05-Jun-2003	Safety Report - Follow-up to a Written Report	BBIND 9030
04-Jun-2003	Request for Information from the FDA	BBIND 9030
29-May-2003	Safety Report - Initial Written Report	BBIND 9030
27-May-2003	Annual Report	BBIND 9030
05-May-2003	Protocol Amendment - Change in Protocol	BBIND 9030
25-Apr-2003	Response to FDA Request for Information – regarding questions sent to Merck on February 10, 2003	BBIND 9030
21-Mar-2003	General Correspondence—Document outlining Merck's clinical program in adolescents	BBIND 9030
12-Mar-2003	Protocol Amendment - Change in Protocol	BBIND 9030
25-Feb-2003	Response to FDA Request for Information—regarding questions sent to Merck on September 23, 2004	BBIND 9030
21-Feb-2003	Safety Report - Initial Written Report	BBIND 9030
19-Dec-2002	General Correspondence – Submission of electronic files required to comply with e-IND pilot program	BBIND 9030

10-Dec-2002	Safety Report - Follow-up to Written Report	BBIND 9030
02-Oct-2002	Protocol Amendment - Change in Protocol	BBIND 9030
21-Aug-2002	Response to FDA Request for Information –Examples of subject study education brochures and recruitment materials	BBIND 9030
20-Aug-2002	General Correspondence – Submission of data in support of aluminum adjuvant	BBIND 9030
22-Jul-2002	General Correspondence – Request for Type B meeting to discuss Phase III clinical development plan and proposed registration database	BBIND 9030
21-Jun-2002	Protocol Amendment - New Protocol (Protocol 016)	BBIND 9030
13-Jun-2002	Annual Report	BBIND 9030
07-Jun-2002	Response to FDA Request for Information regarding questions conveyed to Merck by CBER during April 22, 2002 teleconference	BBIND 9030
02-May-2002	Protocol Amendment - Change in Protocol	BBIND 9030
01-May-2002	Protocol Amendment - New Protocol (Protocol 016, F.U.T.U.R.E. II Study)	BBIND 9030
23-Apr-2002	Protocol Amendment - Change in Protocol	BBIND 9030
19-Apr-2002	General Correspondence-- Merck's plan for developmental and reproductive toxicology testing	BBIND 9030
27-Mar-2002	General Correspondence—Update CBER on Merck's manufacturing plan for Phase III clinical supplies	BBIND 9030
05-Dec-2001	Protocol Amendment - New Protocol (Protocol 011, 012, 013, F.U.T.U.R.E. Study)	BBIND 9030
22-Jun-2001	Protocol Amendment - Change in Protocol	BBIND 9030
01-Jun-2001	Annual Report	BBIND 9030
18-May-2001	General Correspondence- Request for Type B meeting to discuss Phase III clinical Plans	BBIND 9030
05-Apr-2001	Response to FDA Request for Information Response to CBER's comments during November 20, 2000 meeting.	BBIND 9030
21-Dec-2000	General Correspondence – Preliminary Safety/Tolerability Information	BBIND 9030
06-Dec-2000	General Correspondence-- Merck's minutes for the Merck-CBER Post-Phase II meeting held November 20, 2000	BBIND 9030
23-Oct-2000	Response to FDA Request for Information regarding questions received August 15, 2000	BBIND 9030
29-Sep-2000	General Correspondence Request for Type B meeting	BBIND 9030
15-Aug-2000	Protocol Amendment - Change in Protocol	BBIND 9030
06-Jul-2000	General Correspondence Preliminary Safety/Tolerability Information	BBIND 9030
19-Apr-2000	FDA Acknowledgement of Original IND	BBIND 9030
14-Apr-2000	Human Papillomavirus Vaccine (Quadrivalent) (BB-IND 9030) –Protocol 007	BBIND 9030